International Journal of Microbiological Research 10 (3): 87-93, 2019
ISSN 2079-2093

DOI: 10.5829/idosi.ijmr.2019.87.93

# Comparative Efficacy of Prepared Live Oily Adjuvanted and Commercial Inactivated NDV Genotype VIId Vaccines in Egypt

*[1]W.H. EL-Dabae, [2]M.M. EL-Safty and [2]M.F. EL-Sayed*

[1]Microbiology and Immunology Department, Veterinary Research Division,
National Research Center, Dokki, Giza 12622, Egypt
[2]Central Laboratory for Evaluation of Veterinary Biologics, Abbasia, Cairo 11381, Egypt

**Abstract:** Although massive use of different vaccination programs against virulent NDV genotype VIId (Chinese strain) in Egypt, recurrency of outbreaks are continued. In this study, we evaluated the efficacy of two different types of vaccines in the protection against NDV genotype VII infection. Vaccination of live adjuvanted in oil (saponin) and commercial inactivated vaccine in three weeks old SPF chicks revealed neither clinical signs nor post vaccinal reaction after ten days of observation period. Serological immune response of live adjuvanted in oil NDV vaccine was more stronger than inactivated vaccine using HI test. Challenge trial using NDV genotype VIId demonstrating 100% and 90% protection for live adjuvanted in oil and inactivated vaccine respectively. Testing of virus shedding using quantitative real time PCR for live adjuvanted in oil revealed complete absence of virus in tracheal swabs collected on 3, 5,7 and 10 days post challenge whereas, the virus was detected in inactivated vaccine in variable amounts. This study showed that using of live adjuvanted in oil NDV vaccine is better than inactivated vaccine due to strong immune response, better protection level and no virus shedding.

**Key words:** NDV genotype VIId · Live in oil adjuvanted vaccines and inactivated vaccines

## INTRODUCTION

Newcastle disease virus (NDV) is one of the mainly sever and highly contiguous poultry diseases. The causative agent of the disease is NDV, an enveloped virus belonging to the genus *Avulavirus* within the family *Paramyxoviridae* [1,2]. NDV contains non segmented, negative sense, single stranded, RNA genome of 15.2 kb comprising six genes encoding nucleocapsid protein, phosphoprotein, matrix protein, fusion protein, haemagglutinin neuraminidase and RNA dependent RNA polymerase protein [3]. In Egypt, the first record of isolation and molecular characterization of NDV genotype VII was in year 2011 and the phylogenetic analysis revealed that it is velogenic isolate clustered within genotype VII sub genotype d [4]. NDV genotype VIId was isolated in broiler flocks vaccinated previously with Lasota strain experienced respiratory and/or nervous signs with 75% mortality [5]. Also, in year 2014 NDV genotype VIId was reported in different Egyptian localities and Sequence analysis of F-gene revealed velogenic isolate similar to NDV genotype VIId [6].

Vaccination of chickens is the best way to reduce losses resulting from NDV infection. NDV vaccine strains of genotypes I and II are used to control subclinical and clinical forms of the disease during outbreaks [7]. Currently, the most common used conventional ND vaccines; live attenuated Newcastle disease vaccines and inactivated Newcastle disease vaccines [8, 9]. Inactivated vaccines are applicable to individual birds providing longer humeral immunity with high levels of serum antibodies. But, these vaccines are expensive, danger of causing disease when it is not completely inactivated, mineral oil may result in local inflammation at site of injection and do not induce local immunity in the respiratory and digestive tracts although immunity is established rather slowly. While, attenuated live vaccines could induce both local and systemic immunity at low

**Corresponding Author:** Wahid El-Dabae, Microbiology and Immunology Department, Veterinary Research Division, National Research Center, Dokki, Giza 12622, Egypt. E-mail: dr_wahidhussein@yahoo.com.

cost with multiple administration methods but there is risk of reversion to virulent strains with passage from bird to another [10-12].

Adjuvants were mainly used to enhance immune response of inactivated NDV vaccines [13]. To date, it is possible to incorporate substances to modulate the immune response by using live in oil NDV vaccines [14]. The use of live in oil NDV vaccine in one day old broiler chicks provide markedly protection rates with high antibody levels [15]. Using of two different adjuvants with live mesogenic vaccine induced 100% protective and clearly increases HI titers [16].

The present study highlights the efficacy of live in oil adjuvanted and commercial inactivated NDV vaccines in Egypt to control NDV genotype VIId "Chinese strain" circulating in poultry populations.

## MATERIAL AND METHODS

**Vaccine Inoculation in SPF Eggs**

**The Live in Oil Adjuvanted NDV Vaccine:**

- This vaccine was prepared in the Central Laboratory for Evaluation of Veterinary Biologics, Abbasia by serial passage of the well characterized NDV genotype VIId designated as NDV-F278-RLQP-CH-EG (Accession number KM288621.1) in 9-11 day old SPF (Specific pathogen free) eggs (Obtained from Koum-Oshiem Fayoum, Egypt) for thirty five times with initial titration of $10^9$EID (Egg infective dose) /ml [17].
- The final titration of the virus was $10^7$ EID 50/ml [18] based on Reed and Muench [19].
- The allantoic fluid harvested from 35$^{th}$ passage was mixed (volume/volume) with saponin (5% in water) for 30 minutes at room temperature as the best time for reaction then, the mixture (0.2 ml) was inoculated into five 9-11 day old SPF eggs to test the inactivation process by rapid slide haemagglutination test (HA test) [20].

**The Commercial Imported Korean Inactivated NDV Vaccine:**

- This vaccine was randomly selected during the routine work in Central Laboratory for Evaluation of Veterinary Biologics, Abbasia, Egypt.
- This vaccine was undergone for completion of inactivation test. Briefly, 0.2ml of inactivated NDV vaccine was inoculated into five 9-11- day old SPF eggs at least for three passages. The eggs were incubated at 37°C with 40-60% humidity for seven days and candled daily. Embryos dying during first 24 hours are considered non specific. The completion of inactivation of the virus was detected by HA test on the allantoic fluid harvested from inoculated eggs. The inactivation is carried out properly if there is no HA activity in the allantoic fluid.

**Chicken Immunization and Safety of Vaccines:** Forty SPF chicks of three weeks old were used in this study and divided into four groups:

- Ten SPF three weeks old were inoculated with live in oil adjuvanted NDV vaccine with a dose of 0.2ml via subcutaneous route.
- Ten SPF three weeks old were inoculated with double recommended dose of NDV inactivated vaccine.
- Ten SPF three weeks old were kept as positive control and inoculated with 100 µL oculonasal of NDV genotype VIId designated as NDV-B7-RLQP-CH-EG-12 VIId containing $10^6$ EID50/ml.
- Ten SPF three weeks old were kept as negative control.
    - Each chicken group were kept in a separate isolator and monitored for ten days to detect any symptoms which may arise.
    - Organs (Trachea, brain, spleen and kidney) were collected from dead chicks for virus isolation on 9-11- day SPF eggs [21].

**Quality Control of Live in Oil Adjuvanted and Inactivated NDV Genotype VIId Vaccines**

**Immunogenicity:**

- Serum samples were collected from five vaccinated chickens of live in oil adjuvanted vaccine on 3 5,7,14 and 21 days post vaccination and on 28$^{th}$ day post vaccination of inactivated vaccine to measure antibody titer by haemagglutination inhibition test (HI) [22].

**Challenge Trial (Potency) (Protection Level):**

- Challenge trial was applied on the 21$^{th}$ day post vaccination of live in oil adjuvanted vaccine and 28$^{th}$ post vaccination of inactivated vaccine by inoculation of 100 µL oculonasal of NDV genotype VIId designated as NDV-B7-RLQP-CH-EG-12 containing $10^6$ EID50/ml [23].

- Another ten SPF 3 weeks old chicks were kept as positive control group and inoculated as vaccinated challenged group as mentioned above.
- Ten SPF three weeks old chicks were kept as negative control group.
- Each group were kept in a separate isolator and observed for 10 days and the protection % was assessed according to Tizard [24].

**Shedding of NDV Challenged Virus:**

- Tracheal swabs from vaccinated challenged chicks of both vaccines were collected on 3rd, 5th, 7th and 10th days post challenge [25] and tested by qRT- real time PCR [26].

**Sterility of Vaccines:**

- Samples from both vaccines were inoculated into nutrient agar and thioglycolate broth media then incubated at 37°C for 7 days to detect bacterial contamination. Other samples were cultured on Sabaroud agar media and incubated at 25°C for 14 days to observe fungal contamination. Both vaccines were tested for mycoplasma by culturing on PPLO agar and incubated for 14 days at 37°C [27].

**Purity of Vaccines:**

- Purity of both vaccines was tested by HA test to exclude any Haemaggluitinating viruses other than NDV as avian influenza or adeno virus using specific antisera for each virus [27].

**Identity of Vaccines:**

- Identity of both vaccines was tested by collection of serum samples from vaccinated chickens on 21th day and 28th day post vaccination of live in oil adjuvanted and inactivated vaccines respectively and tested by HI test using homologous NDV antigens [28].

## RESULTS

**Results of Vaccine Inoculation in SPF Eggs:**

- Inoculation of SPF eggs with 35th passage of NDV genotype VIId with saponin (Live adjuvanted vaccine) revealed deaths of all egg embryos 2nd day post inoculation and HA positive by rapid HA test. While, negative agglutination of inactivated vaccine was observed after three passages in SPF eggs indicating the virus was completely inactive.

**Results of Vaccine Immunization and Safety in SPF Chicks:**

- After ten days of observation period neither clinical signs nor post vaccinal reaction was detected for both live adjuvanted in oil and inactivated NDV vaccine. Whereas, 100% of positive control group died after five days post vaccination with successful of virus isolation on 9-11 day SPF eggs and the isolated virus showed HA titer of 7 $\log_2$ and HI titer of 10 $\log_2$. No mortalities appeared on negative control group.

**Results of Quality Control of Live in Oil Adjuvanted and Inactivated NDV Genotype VIId Vaccines**

**Immunogenicity:**

- Testing of serum antibodies collected from vaccinated chicks of live adjuvanted in oil vaccine showed strong immune response and high titer of antibody. The mean HI titer expressed as $\log_2$ was 7, 8, 11, 12 and 12 at interval times 3, 5,7,14 and 21 days post inoculation respectively.
- Testing of serum antibodies collected from vaccinated chicks of inactivated vaccine revealed weak immune response. The mean HI titer was 6 $\log_2$ on 28th day post vaccination.

**Challenge Trial (Protection Level):**

- Challenge trial revealed 100% and 90% protection of vaccinated chickens for live adjuvanted in oil and inactivated vaccine respectively after 10 days observation period. All chicks in positive control group died after five days post challenge.

**Shedding of NDV Challenged Virus:**

- Real time RT- PCR revealed no virus shedding of live adjuvanted in oil vaccine in tracheal swabs collected from vaccinates chickens on 3rd, 5th, 7th and 10th days post challenge (Figure-1). Whereas, NDV was detected in variable amounts of inactivated vaccine (Figure-2).

**Results of Sterility of Vaccines:**

- Both vaccines were free from any aerobic, anaerobic, fungal and mycoplasma contamination.

**Results of Purity of Vaccine:**

- Live adjuvanted in oil and inactivated vaccines showed Negative HA expect NDV.

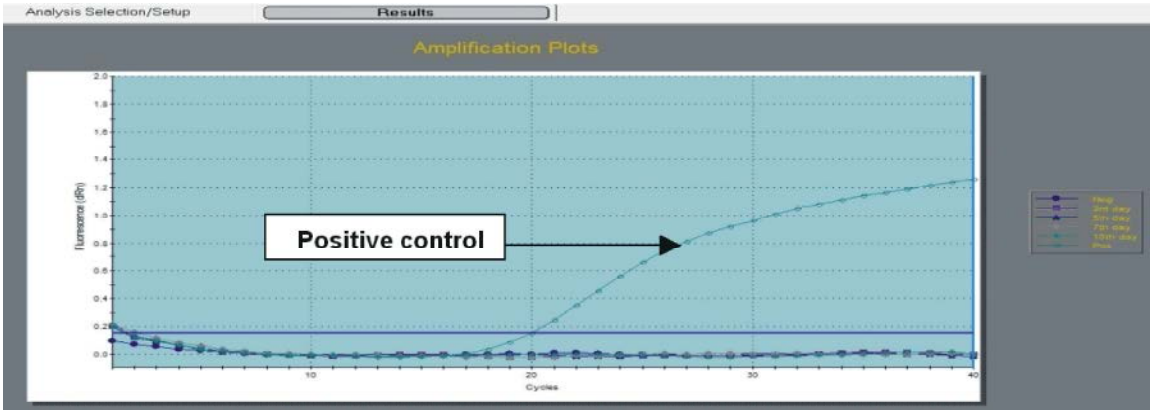


Fig. 1: qRT-PCR amplification curve showing complete absence of NDV in tracheal swabs from vaccinated chickens of live adjuvanted in oil vaccine on 3, 5, 7 and 10 days post challenge in comparison with positive control (challenge virus) with cycle threshold (c.t) 17.

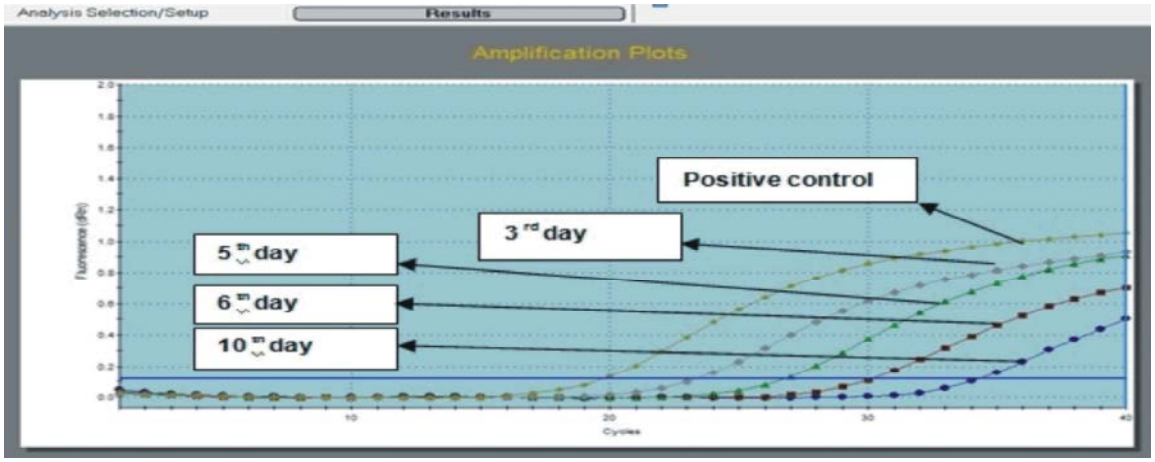


Fig. 2: qRT-PCR amplification curve showing detection of NDV RNA from inactivated vaccine fold serial of the challenge virus (positive control c.t 21, 3rd day c.t 25, 5th day c.t 28, 7th day c.t 31 and 10th day c.t 35).

**Results of Identity of Vaccines:**

- Live adjuvanted in oil and inactivated vaccine showed antibody titer of 12 $\log_2$ and 6 $\log_2$ respectively by HI test indicating identity of both vaccines.

## DISCUSSION

In the current study, we evaluated the efficacy of two different types of NDV genotype VIId vaccines. We use saponin in live in oil adjuvanted NDV vaccine. Saponin has been known to have adjuvant properties since 1920 [28]. Normally, oil adjuvant used with inactivated vaccines to enhance immunogenicity but this study used the saponin as oil adjuvant with propagated NDV after 35th passages for 30 minutes incubation at room temperature. This live in oil vaccine induced 100% protection when evaluated in SPF chicks. Additionally, it induced strong immune response post vaccination with no shedding after challenged with genotype VIId of NDV (Figure 1). While the commercial inactivated vaccine used in this study induced low antibody titer and sheds the virus in variable amounts (Figure 2). In accordance with this study, Peleg *et al.* [29] investigated the use of live in oil ND vaccine for immunization of chickens at different ages. They concluded that live in oil vaccines were shown to be 30-50 times more effective in efficacy tests than either the same vaccines reconstituted in water or killed vaccines.

The use of live in oil NDV vaccine in one day old broiler chicks in comparison with killed in oil ND vaccine provide markedly protection rates and antibody levels although live ND vaccine containing $10^6$ embryo lethal dose 50 whereas, killed in oil ND vaccine containing $10^{8.4}$

embryo lethal dose 50 [15]. As well, mixing of mucosal immune adjuvant compounds with attenuated ND vaccine for vaccination of 7 day old chickens improved humoral and mucosal immunity with 100% protection after 7 days from challenge with virulent NDV [14]. Oral administration of leaf saponin intranasal with live ND vaccine in chickens revealed high HI titer of live ND vaccine and also improve cellular immune response (Lymphocyte proliferative test, IgA and intestinal intraepithelial lymphocytes) in chickens [30]. In Egypt, vaccination of chickens with live NDV vaccines (Lasota and clone 30) with *Nigella sativa* oil revealed 73.33% protection at 21 days post vaccination and 26.66% protection at 42 days post vaccination while, vaccination with Lasota only induced 66.66% and 40% at 21- and 42- days post vaccination respectively [31]. The live in oil vaccine has both the effect of live and killed vaccines as non adjuvanted vaccine may fail to establish immunity in the presence of antibody because of virus neutralization but adjuvanted vaccine would induce active immunity even in the presence of antibody due to slow and persistent release of the virus from the oil environment [16].

Live in oil vaccine in enhance the escape of infective live virus from the trapping oil environment and thus initiation of infection and replication of the virus in the various tissues and organs. This triggering of the immune system by live virus at a very early stage of the immunization is assumed to be an effective event. It manifests itself later on when followed by boosters of the remaining virus which is killed in the oil and released from it continuously at a certain rate, behaving then as a killed in oil vaccine [29]. The use of adjuvants in live vaccine could improve the efficacy and lead to better management of the antigen load per vaccine dose and this also improve the safety of the vaccine as the possible adverse reactions observed after delivery of live infectious vaccines could be lowered. Moreover, the risk of reversion to virulence that has already been observed in avian species would be reduced [32]. The use of adjuvants reduces the number of low or not responding chickens and therefore decreases the possible reservoir for the disease [33]. Adjuvanted vaccine performance could also compensate the decrease of antigenic load in case of impaired vaccine delivery or formulation, a possible virucidal effect of storage or inappropriate resuspending conditions [34].

Overall, this study supports using of live adjuvanted in oil NDV vaccine than inactivated vaccine for the protection of poultry populations against NDV genotype VIId strain.

## REFERENCES


1. Alexander, D.J., 2011. Newcastle disease in the European Union 2000 to 2009. Avian Pathol., 40: 547-558.
2. Ganar, K., M. Das, S. Sinha and S. Kumar, 2014. Newcastle disease virus: current status and our understanding. Virus Res., 184: 71-81.
3. Alexander, D.J., E.W. Aldous and C.M. Fuller, 2012. The long view: a selective review of 40 years of Newcastle disease research. Avian Pathol., 41: 329-335.
4. Radwan, M.M., S.F. Darwish, I.M. El-Sabagh, A.A. El-Sanousi and M.A. Shalaby, 2013. Isolation and molecular characterization of Newcastle diseasevirus genotypes II and VIId in Egypt between 2011 and 2012.Virus Genes, 47: 311-316.
5. Abdel-Glil, M.Y., S.K. Mor, T.A. Sharafeldin, R.E Porter and S.M. Goyal, 2014. Detection and characterization of Newcastle disease virus in formalin-fixed, paraffin-embedded tissues from commercial broilers in Egypt. Avian Dis., 58(1): 118-123.
6. Awad, A.M., M.E. Sedeik and A.A. Abdelkariem, 2015. Isolation, Molecular characterization and Pathotyping of Newcastle disease virus from field outbreaks among broiler flocks in Egypt from 2014-2015. Int. J. Current Res., 7(2): 12925-12934.
7. Perozo, F., P. Villegas, C. Estevez, R. Alvaradoi, L.B. Purvis and E. Saume, 2008. Avian adeno-associated virus based expression of Newcastle disease virus hemagglutinin-neuraminidase protein for poultry vaccination. Avian Dis., 52(2): 253-259.
8. Huyge, K., K. Van Reeth, T. De Beer, W.J.M. Landman, J.H.H. Van Eck, J.P. Remon and C. Vervaet, 2012. Suitability of differently formulated dry powder Newcastle disease vaccines for mass vaccination of poultry. Europ. J. Pharmaceutics and Biopharm., 80: 649-656.
9. Zhao, W., H. Hu, L. Zsak, Q. Yu and Z. Yang, 2013. HN gene C-terminal extension of Newcastle disease virus is not the Determinant of the Enteric Tropism. Virus Genes, 47(1): 27-33.
10. Tretyakova, I., I.S. Lukashevich, P. Glass, E. Wang, S. Weaver and P. Pushko, 2013. Novel vaccine against Venezuelan equine encephalitis combines advantages of DNA immunization and a live attenuated vaccine. Vaccine, 31(7): 1019-1025.

11. Zhao, X.J., Y.P. Fan, D.Y. Wang, Y.L. Hu, L.W. Guo, S.L. Ruan, J. Zhang and J. Yuan 2011. Immunological adjuvant efficacy of glycyrrhetinic acid liposome against Newcastle disease vaccine. Vaccine, 29: 9611-9617.
12. Wu, Y.M., F.J. Zhao, X.H. Zhang, J. Yu, W.M. Gu, S.Q. Liu, T. Zeng, Y. Zhang and S. Wang, 2011. Enhanced immune response and protective efficacy of a Treponema pallidum Tp92 DNA vaccine vectored by chitosan nanoparticles and adjuvanted with IL-2. Human Vaccines, 7(10): 1083-1089.
13. Yin, J., H. Jin, Y. Kang, C. Xiao, L. Zhao, X. Li, Z. Ding, F. Yang, Q. Zhu and B. Wang, 2006. Efficacy of modified levamisole adjuvant on inactivated virus vaccine. Viral. Immunol., 19: 525-535.
14. Zhang, X., X. Zhang and Q. Yang, 2007. Effect of compound mucosal immune adjuvant on mucosal and systemic immune responses in chicken orally vaccinated with attenuated Newcastle-disease vaccine. Vaccine, 25: 3254-3262.
15. Samina, I., Y. Khinich, B. Gutter, A. Michael and B.A. Peleg, 1999. Day-old vaccination with live-in-oil vaccines: Newcastle disease (ND) and infectious bursal disease (IBD) in chicks and ND in turkey poults. Avian Pathol., 28: 73-78.
16. Roy, P., A.T. Venugopalan and A. Koteeswaran, 1999. Efficacy of live adjuvanted mesogenic Newcastle disease vaccine in chicken. Vaccine, 17: 2674-2676.
17. Allan, W.H., 1973. The stability of Newcastle disease virus vaccines in copper pipes. Veterinary Record, 93: 16-448.
18. Villegas, P. and H.G. Purchase, 1989. Titration of Biological suspension In Purchase, H.G., H. Lawrence, C. H. Domermuth and E. James, Pearson, (eds). A Laboratory manual for the isolation and identification of Avian pathogens. American Association of Avian Pathologists, University of Pennsylvania, New Bolton Center, Kennett Square, PA. 19348-1692:186-191.
19. Reed, L.J. and H. Muench, 1938. A simple method of estimating fifty percent endpoint. American Journal of Hygiene, 27: 493-497.
20. OIE, 2012. Newcastle disease. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 2.3.14. http://www.oie.int/ international-standard-setting/terrestrial-manual/access-online.
21. Burleson, F.G., T.M. Chambers and D.L. Wiedbrauk, 1994. Virology a laboratory Manual. Academic Press, Harcourt Brace Jovanovich, Publishers, New York.
22. OIE, 2009. Manual of diagnostic tests and vaccines for terrestrial animals: mammals, birds and bees. In: Biological Standards Commission, World Organization for Animal Health, Paris, pp: 576-589.
23. Allan, W.H., J.E. Lancaster and B. Toth, 1978. Newcastle Disease Vaccines Their Production and Use. FAO Animal Production and Health Series No.10. Food and Agriculture Organization of the United Nations, Rome.
24. Tizard, I.R., 1996. Veterinary Immunology. 5th Ed. W.B. Saunders Company, Philadelphia, pp: 251-263.
25. Miller, P.J., D.J. King, C.L. Afonso and D.L. Suarez, 2007. Antigenic differences among Newcastle disease virus strains of different genotypes used in vaccine formulation affect viral shedding after a virulent challenge. Vaccine, 25(41): 7238-7246.
26. Wise, M.G., D.L. Suarez, B.S. Seal, J.C. Pedersen, D.A. Senne, D.J. King, D.R. Kapczynski and E. Erica Spackman, 2004. Development of a Real-Time Reverse-Transcription PCR for Detection of Newcastle Disease Virus RNA in Clinical Samples. J. Clinic. Microbiol., 42(1): 329-338.
27. FAO, 2002. Laboratory Manual for the Small-Scale Production and Testing of I-2 Newcastle Disease Vaccine RAP publication (2002/22); ISBN 974-7946- 26-2.
28. Anders, S. and C.C. John, 1998. Uptake and adjuvant activity of orally delivered saponin and ISCOM vaccines. Advan. Drug Delivery Rev., 34: 321-338.
29. 29-Peleg, B., I. Samina and J. Brenner, 1993. Immunization of chickens with live Newcastle disease vaccine adjuvanted with oil. Vaccine, 11(10): 1074-1076.
30. Zhai, L., Y. Li, W. Wang, Y. Wang and S. Hu, 2011. Effect of oral administration of ginseng stem-and-leaf saponins (GSLS) on the immune responses to Newcastle disease vaccine in chickens. Vaccine, 29: 5007-5014.
31. Hussein, A.S. and H.M. Madbouly, 2005. Vaccination of chickens with live Newcastle disease virus vaccines adjuvanted with Nigella sativa oil. Beni-Suef Vet. Med. J., 15(2): 123-126.
32. Guy, J.S., H.J. Barnes and L. Smith, 1991. Increased virulence of modified-live infectious laryngotracheitis vaccine virus following bird to bird passage. Avian Dis., 35: 348-355.

33. Cavanagh, D., 2003. Severe acute respiratory syndrome vaccine development: experiences of vaccination against avian infectious bronchitis coronavirus. Avian Pathol., 32:567-582.

34. Deville, S., J. Ben Arousa, F. Bertranda, V. Borisovb and L. Dupuisa, 2012. Efficacy of intranasal and spray delivery of adjuvanted live vaccine against infectious bronchitis virus in experimentally infected poultry. Procedia in Vaccinology, 6: 85-92.