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REFORMULATION OF NEWCASTLE DISEASE VACCINE USING CHITOSAN NANOPARTICLES IN BROILER

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Abstract

Newcastle disease (ND) is an exceptionally infectious viral disease of poultry brought about by pathogenic strains of the Newcastle disease (ND). This study aimed to increase the time of immune response to the Newcastle vaccine by using chitosan nano-particles and protect ND vaccine from degradable. A live-ND vaccine (strain La Sota) against NDV carrying chitosan nanoparticles were created utilizing an ionic crosslinking technique. The NDV-CS-NPs vaccine were produced with good morphology, A 60 chicks (Breed: Rose 308, origin: Belgium) were weighed at hatching 39.3gm were used in this study. Blood samples were collected randomly in 5 days old for measuring of maternal immunity against Newcastle virus (NDv) using ELISA test (Indirect method). Measuring IgG level for detection immune response at 17 and 24 day old chicks. Chitosan nanoparticles were produced in good morphology and better dispersion. The reformulation of ND vaccine using chitosan nanoparticles that give highly immune response in compared with ND vaccine (La Sota), The immunological parameters were induced in this study (IgG) showed highest level of IgG in experimental one was 688.57 at 17day old chicks and 1207.94 at 24 day old chicks. Conclusion: The present results approve the Advancement of mucosal Newcastle disease vaccine carrying chitosan nanoparticles encouraging further studies in the future. NDV-CS-NPs vaccine triggered better protections of vaccinated chickens contrasted with the live NDV vaccine strain La Sota.

Key words: Newcastle disease (ND), chitosan nanoparticles,

Introduction

Newcastle disease (ND) is a viral disease of poultry caused by a single-strand, non-segmented, negative-sense RNA virus known as Avian paramyxovirus 1 (APMV-1). (Cattoli, 2011). Newcastle disease virus NDV have ability to infected a wide variety of avian species and the pathogenicity of NDV through species is variable , poultry are most susceptible to NDV, with high morbidity and mortalities was seen in broiler and layer flocks (Wajid *et al*., 2016). Nanotechnology has been going too much consideration since 1980s and has been adjusted into numerous designing fields, for example, gadgets, mechanical, biomedical and space building , specifically, nanotechnology has prompted to the critical advance in a biomedical field, for example, controlled medication/ quality delivery (Penaloza *et al*., 2017) On the other hand, the application of nanotechnology for monitoring and control of biological systems has recently been determined by the National of Health (NIH) as nano-medicine, however, the strategy of Nanoparticle delivery plays a significant impact on global Pharmaceutical planning and marketing, polymeric nanoparticles are used to control the drug release, to improve the dissolution of poorly soluble drugs in addition to improve the bioavailability of degradable substances such as protein (Rajalakshmi *et al*., 2014). Study by Kia *et al*., (2012), revealed that the amino and carboxyl groups in the chitosan molecule interact with glycoprotein in mucus to form a hydrogen bond, which produces an adhesive effect, as mucoprotein in mucus is positively charged, chitosan and mucus are attracted to each other to prolong the *in vivo* retention and release time of drugs and to improve drug bioavailability.

Materials and Methods

Preparation of Chitosan nanoparticles (CS-NPs)

Chitosan nanoparticles were prepared by ionic gelation method. Cross linking of chitosan solution with Tripolyphosphate (TPP) (Liang *et al*., 2017).

Particle Size Distribution of CS-NP

The particle size of CS-NPs was distributed in a range of 0.2-350µm, The size distribution of chitosan

nanoparticle suspension was analyzed using particle size analyser in nanotechnology center/university of technology.

Chitosan nanoparticles yield and recovery percent

Chitosan Nanoparticles (CS-NPs) yield and recovery percent were counted after converted Chitosan (total amount used in this study) in to Chitosan Nanoparticles according to equation.

recovery percent =

$$\frac{Chitosan\ Nanoparticles\ yield}{total\ amount\ of\ chitosan} \times 100\%$$

Preparation of Newcastle vaccine-chitosan nanoparticles (NDv-CS-NPs):

The Newcastle vaccine-chitosan nanoparticles (NDv-CS-NPs) nanoparticles were prepared using an ionic cross linking method (Zhao *et al*., 2012), was formed in pharmacology department/College of Veterinary medicine at University of Baghdad as followed: 2.5 milliliters of NDV solution were added drop by drop to 5ml of chitosan solution under magnetic stirring. 2.5ml of TPP solution was added to the above solution under magnetic stirring at room temperature. The chitosan-NDV nanoparticles were separated by centrifugation at 10,000g/min for 30min at 4°C and the supernatant was discarded. The Newcastle vaccine-chitosan nanoparticles were washed with distilled water. These nanoparticles were named as NDV-CS-NPs.

Standardization of Newcastle vaccine-chitosan nanoparticles

The chitosan nanoparticles (CS-NPs) encapsulated Newcastle disease virus was examined with number of parameters in order to identity its physiochemical properties as following:

A. Determination of absorbance curve:

The absorbance curve and λ peak of NDV-CS-NPs where scanned by UV visible spectrophotometer in sequence of wavelength from 200-900nm at 1% 25°C (Latha *et al*., 2012). In pharmacology department/ College of Veterinary Medicine/University of Baghdad.

B. Osmotic tolerance of NDV-CS-NPs

Osmolality tolerance of NDV-CS-NPs was challenge with different hypo-hyper tonic solution 1, 0.975, 0.95, 0.925, 0.9, 0.875, 0.85% NaCl in 25°C (Su *et al.*, 2015). The procedure of NDV-CS-NPs Osmotonicity tolerance assessment : prepared 3.5% and added stock 0.2 ml in each 3 ml of NaCl tonic solution with gentle mix. The NDV-CS-NPs number was estimated by spectrophotometer at 354 nm at zero and after one hour incubation and calibrate in prior formation standard curve of NDV-CS-NPs numbers and expressed as linear fitted curve.

C. pH tolerance

The assessment of NDV-CS-NPs tolerance in pH changes was measured by pH meter in pharmacology department/College of Veterinary Medicine at Baghdad University, The stander concentration of 10 mg/ml of NDV-CS-NPs suspension in normal saline exposed to different pH value (4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 and 10) to estimate if the NDV-CS-NPs are influenced by exposure to different pH value simulation environment of chicken Gut. pH was measured at zero and after one hour with spectrophotometer at 354 nm.

D. Microscopic identity of Newcastle vaccine-chitosan nanoparticles

The NDV-CS-NPs sample (2ml) were examined in scanning and transmitted techniques. The micrograph and procedure was performed in Abcam Laboratory, Los Alamos.com, USA, for E.M. imaging scan type and transmission scattering technique (Sun *et al*., 2016).

E. Entrapment efficiency

The percentage of entrapped NDV-CS-NPs from CS-NPs was measured after complete loading. The methodological protocol of NDV-CS-NPs entrapment was done according to (Senthilkumar *et al*., 2012). The samples were centrifuged at 3000rpm and separated NDV-CS-NPs globule and supernatant , the present yield was calculated by using Equation

Entrapment % =

$$\frac{amount\ of\ total\ vaccine - amount\ of\ free\ vaccine}{amount\ of\ total\ vaccine} \times 100$$

Experimental design

Study subjects consisted of 60 broiler. in this study the chicks divided randomly into 6 groups; each group contained 12 chicks. Intranasal vaccine at 7 days old depend on maternal immunity titer, the vaccination where done as follow:

- First group : control.

- Second group : Intranasal vaccine with NDV (La Sota) (EID_{50} : $10^{9.5}$).

- Third group : Intranasal vaccine with NDV-CS-NPs (EID_{50} : $10^{5.5}$).

- Fourth group : Intranasal vaccine with NDV-CS-NPs (EID_{50} : $10^{6.5}$).

- Fifth group : Intranasal vaccine with NDV-CS-NPs (EID_{50} : $10^{7.5}$).

- Sixth group : Intranasal vaccine with NDV-CS-NPs (EID_{50} : $10^{8.5}$).

Blood samples were collected randomly from 10 chicks of each group at 12 and 17 days old for immunological test (ELISA).

Results

Chitosan nanoparticles standardization

Chitosan nanoparticles yield and recovery percent

The amount of chitosan nanoparticles yield from total amount of chitosan used in this study and the recovery percent were calculated in the table 1.

Estimation Chitosan nanoparticles size

Chitosan nanoparticles suspension (CS-NPs)results were displayed particles size distribution in fig. 1. The CS-NPs ranged between 82.35 ± 4.2nm and 109.14 ± 14.2 nm (90.26 ± 9.58nm) at wave length scanned between 0.2 - 350nm.

Standardization of Newcastle vaccine-chitosan nanoparticles

Absorbance curve

The results of Newcastle vaccine-chitosan nanoparticles (NDv-CS-NPs) standardization , where examined by UV visible spectrophotometer. The Absorbance curve and λ peak was 354nm as shown in the fig. 2.

Osmo-tolarence NDv-CS-NPs

The osmolality tolerance of NDV-CS-NPs was estimated by spectrophotometer at 354nm at Zero time and after one hour show significant ($p<0.05$) changes between (0.5-0.8) in compared with other osmolality changes points as shown in the fig. 3.

pH tolerance challenge NDv-CS-NPs

The tolerance of pH of NDV-CS-NPs was measured at zero time and after one hour by UV visible spectrophotometer at 354nm the results show significant changes between (6-12) in compared with other pH tolerance points as shown in the fig. 4.

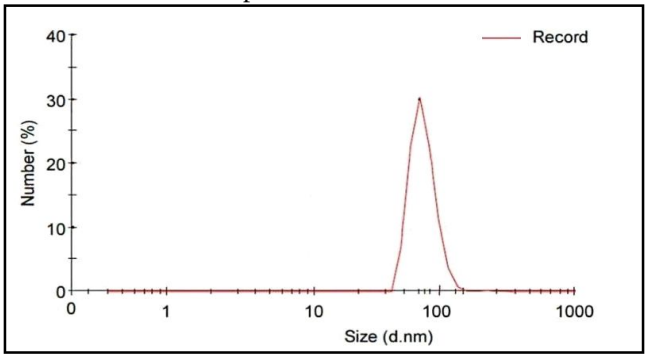

Fig. 1: Particle size distributions of Chitosan Nanoparticles .

Table 1: Show the results of chitosan nanoparticles yield and recovery percent.

Total amount of Chitosan	Chitosan nanoparticles yield	recovery percent
40 mg	22.83 mg ± 9.73	57.075 ± 23.46

Chitosan Nanoparticles Entrapment of ND vaccine percent

The result of entrapment percentage of entrapped NDV-CS-NPs from CS-NPs according to Equation of entrapment were 43.6% entrapment of NDV-CS-NPs.

Electron micrograph profile

The microscopic depiction of NDV-CS-NPs in scanning electron microscope and transmission electron microscope scanning. The morphology of NDV-CS-NPs were prepared had a regular round shape and good dispersion, but it did not have adhesion or subsidence damage, the measurement of these particles showed a distribution from 214.2 to 524.9nm with an average particle size of 327.2nm, the results shown in the following fig. 5.

Determination of viral content in the NDV-CS-NP

The result of viral titer of Newcastle disease virus in NDV(La Sota)-CS-NPs was 109.5 EID50 /0.1 ml, which met the manufacturing standards for the attenuated live ND vaccine as required by the Veterinary Biological Product Rules of the People's Republic of China (≥105 EID50).

Evaluation of maternal immunity against ND:

Ten serum samples out of (60) chicks (before division

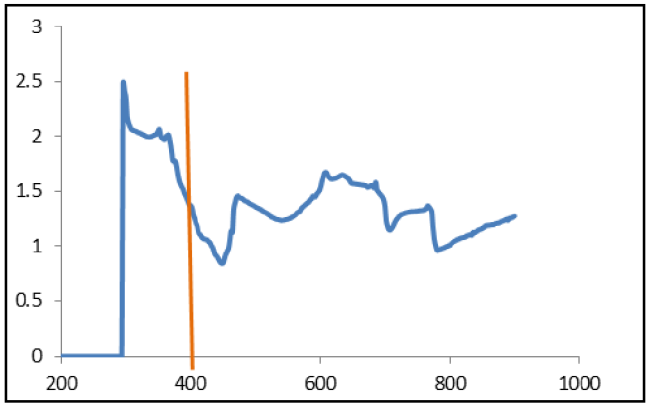

Fig. 2: The absorbance curve and ë peak of NDV-CS-NPs where scanned by UV visible spectrophotometer .

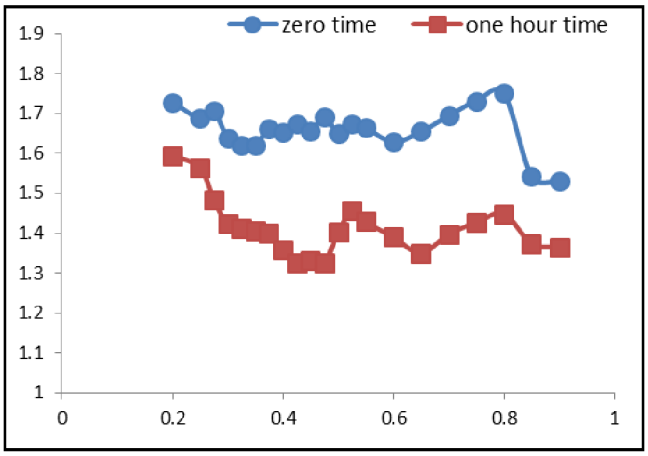

Fig. 3 : Osmolality tolerance of NDV-CS-NPs measured at zero and after one hour by UV visible spectrophotometer.

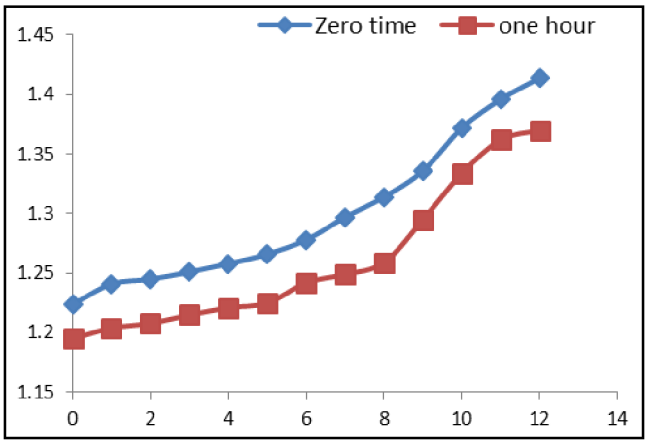

Fig. 4: pH tolerance of NDV-CS-NPs measured at zero and after one hour by UV visible spectrophotometer.

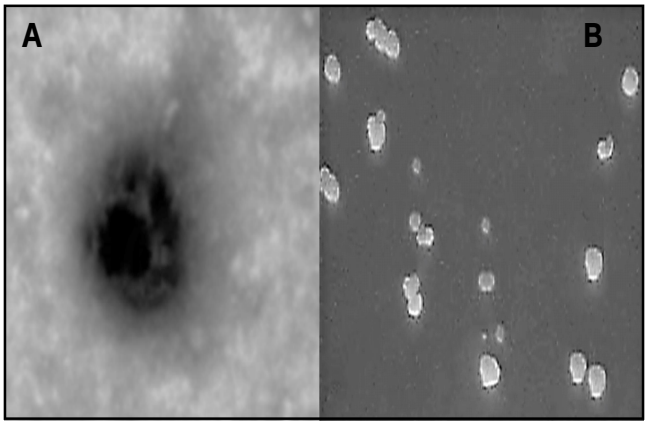

Fig. 5: Electron micrograph of NDV-CS-NPs A. Transmission depiction of NDV-CS-NPs at 200nm. B. Scanning depiction of NDV-CS-NPs at 500nm.

in to groups) were evaluated in experimental one by using ELISA test and the results revealed good immune response immunity to NDV at 5 day old chicks and found the Mean value ± SE was (2248.36 ± 221.43).

Table 2: The means value of antibody titer (IgG) at 17 and 24 day old of chicks against Newcastle disease viral - vaccine (Mean ± SE).

Group	antibody titer (IgG) at 17day old	antibody titer (IgG) at 24day old
Group 1	387.74 ± 11.79 d	180.46 ± 12.06 e
Group 2	513.16 ± 6.70 c	815.15 ± 15.89 d
Group 3	533.70 ± 12.85 b	889.96 ± 6.85 c
Group 4	573.52 ± 13.23 c	989.79 ± 5.47 b
Group 5	688.57 ± 5.11 a	1207.94 ± 26.13 a
Group 6	587.54 ± 4.99 c	1001.70 ± 10.57 b
LSD value	27.779 **	41.243 **
P-value	0.01	0.01

** ($P \leq 0.01$).Means having with the different letters in same column differed significantly.

Measurement of Immunoglobulin G

The results of the present study showed a significant differences ($P \leq 0.01$) between all vaccinated groups in compared with control group in IgG concentration in different periods (17 and 24) days old, at 17 days old chicks showed the highest mean titer in G5 was (688.57 ± 5.11) followed by G6, G4, G3, G2 which were (587.54 ± 4.99, 573.52 ± 13.23, 533.70 ± 12.85, 513.16 ± 6.70) respectively, while the lowest concentration was G1 (control) was (387.74 ± 11.79).

While, the results of IgG concentration at 24 days old chicks displayed the highest mean titer in G5 was (1207.94 ± 26.13) subsequently G6, G4, G3, G2 which were (1001.70 ± 10.57, 989.79 ± 5.47, 889.96 ± 6.85, 815.15 ± 15.89) respectively, while the lowest concentration was G1 (control) was (180.46 ± 12.06). The results showed in the table 2.

Discussion

A total of 40mg of chitosan was used in the present study, the results reported amount of Chitosan Nanoparticles (CS-NPs) yield were 22.83 ± 9.73mg with recovery percent 57.075 ± 23.46 which indicated that the preparation processes by ionic gelation method depend on cross linking charges between Chitosan (CS) (positive charge) and tripolyphosphate (TPP) (negative charge) were acceptable and the appearance of CS-NPs was milky color with gel consistency, this in agreement with Khameneh *et al.*, (2014), Shariatinia and Jalali (2018) they were explained that the Nanoparticles formation largely depend on their physicochemical properties, such as size, opposite molecule charges and surface characteristics. Newcastle disease vaccine had added to chitosan solution drop by drop at room temperature with stirring then added tripolyphosphate sodium after that it combined and form NDV-CS-NPs vaccine , the

viral content of Newcastle disease vaccine in NDV-CS-NPs was 109.5 EID50/0.1ml that used in the study, these results in compatible with Zhao *et al.,* (2012) they were explained that the preparation of NDV-CS-NPs and the optimal combination for NDV-CS-NPs was tripolyphosphate sodium to NDV/CS solution a ratio of 1:2.

The results of the absorbance curve of and λ peak of NDV-CS-NPs were determined, these in agreement with Krishnaveni and Priya (2014) who were explained that the peak of NDV-CS-NPs was due to the presence of amino group in chitosan The osmolality tolerance of NDV-CS-NPs vaccine was range from 0.5 to 0.8 of NaCl gradients concentrations was occur presumably due to the membrane integrity were referred to rheological activity of chitosan nanoparticles effect the ingredient concentration by time scale; measured at zero time and after one hour, The pH tolerance of NDV-CS-NPs vaccine when hydrogen ions enter into the tail of phospholipid causing destruction and cause partial coefficient hyper osmosis and decrease in fluidity and elasticity these have agreement with Kulthe *et al.,* (2012) who were explained that due to progressive amine protonation of pendant groups the Chitosan soluble at high pH values approximately 6 or less and become positive charge lead to polycataionic behavior.

The result of serum sample in this experimental before division into groups for assessment maternal immunity to NDV finding was in agreement with Waheed *et al.,* (2013) who were found that the multiple vaccination against ND in different routes to breeders flock in rearing period as well as in production period, several types of vaccinal strains against ND like (B1, La Sota and oil immersion) all those were used in vaccination before lying. In addition, Agrawal *et al.,* (2016) they were reported that the cumulative effect of Ab production resulted from several times of vaccination reach to higher titers, thus the eggs will received a large amount of Ab from their own breeders and this represented in hatched chicks.

In this experimental used a different concentrations of NDV-CS-NPs vaccine and choose the better IgG level, the results of this experiment were determined the optimum immunization dose, this in agreement with Boyoglu *et al.,* (2009) they were reported that the NDV-CS-NPs vaccine is important in the vaccine efficacy trials for the effectual stimulation of immune responses in chickens.

The optimum dose of the NDV-CS-NPs vaccine containing 107.5 EID50/0.1ml was chosen in the vaccine efficacy trial in the study, the result in group 5 for IgG detections was (688.57 ± 5.11) at 17 days old chicks and (1207.94 ± 26.13) at 24 days old chicks, as shown in the table 2, while the result of ordinary (La Sota) vaccine in group 2 was (513.16 ± 6.70) at 17 days old chicks and (815.15 ± 15.89) at 24 days old chicks, respectively. As shown in the table 2, this in agreement with Bwala *et al.,* (2011) who were explained that the better immune responses were induced in chickens immunized with the NDV-CS-NPs vaccine in compared to chickens immunized with the normal live NDV vaccine (La Sota).

Conclusions

We concluded that present results approve chitosan nanoparticles measured by particles size distribution was 90.26nm. Standardization of NDV-CS-NPs vaccine in absorbance curve and λ peak was 354nm and osmolality and pH tolerance were measured, entrapment percentage was 43.6%. viral content in NDV-CS-NPs vaccine was $10^{9.5}$ EID_{50} /0.1 ml. NDV-CS-NPs was scanned by scanning electron microscope and transmission electron microscope scanning. Maternal immunity against ND in experimental one was 2248.36, while in experimental two 3737.90 .Immunological parameters were induced in this study (IgG) showed highest level of IgG in experimental one was 688.57 at 17 day old chicks and 1207.94 at 24 day old chicks.

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