RESEARCH ARTICLE Agricultural Scinece Digest, Volume 39 Issue 4: 347-352 (October-December 2019)

Immunological Effects of a Single Dose of PLGA Nanoparticles Encapsulated Peptide in Broilers in Comparison to Traditional Vaccines against Infectious Bursal Disease

S.H. Al-Rubaee, T.S. Al-Azawi[1] and A.A. Taha[2]

10.18805/ag.D-171

ABSTRACT

Infectious bursal disease is a viral poultry disease that causes economic losses. This study aimed to evaluate the white blood cells count (WBCs count), differential count, antibody (Ab) titration against IBDV and interferone gamma (INF-γ) responses in broiler administered traditional and prepared nanovaccines. A total of 98 broiler chicks (Ross 308) were used to evaluate the immunological responses. They were devided into G1(Control); G2- (Traditional vaccine); G3(PLGA nanoparticle); G4(160 µg of peptide loaded PLGA); G5 (80 µg of peptide loaded PLGA); G6(40 µg of peptide loaded PLGA) and G7(20 µg of peptide loaded PLGA) by oral administration. At day 19 of broiler age, the chicken was administered orally with traditional and prepared nanovaccines and the blood was collected for Ab titration at day 10(for maternal immunity), day 29 and day 42 of broiler age. At the end of experiment, whole blood was collected for WBCs and differential count and for INF-γ level. The results indicated that there were no significant ($p \geq 0.05$) differences among experimental groups in WBCs count while there were significant $p \leq 0.05$) increase in lymphocyte count in G3 (NPs) in compared with G2(traditional vaccine). Also there was a significant ($p \leq 0.05$) increase in heterophil count in G4 (NPs + 160µg peptide) as compared to control. For Ab titration, at day 29, data indicated significant ($p \leq 0.05$) increase in G3 and G7 as compared to G2 while at day 42, there was a significant ($p \leq 0.05$) increase in each of G3,G4,G5 and G6 groups in compared to control and traditional vaccine. For INF-γ level, G4 recorded significantly ($p \leq 0.05$) the highest level as compared to other groups. These immunological responses were explored in order to evaluate the prepared nanovaccine for IBD in broiler.

Key words: Ab Titration, Brioler, INF-γ, Nanovaccine, PLGA nanovaccine, Wbcs Count.

INTRODUCTION

Infectious bursal disease virus (IBDV) is the causative agent of a highly contagious disease in young chickens, and has a major impact on the economy of the poultry industry worldwide. IBDV multiplies rapidly in developing B-lymphoid cells, resulting in the destruction of the precursors of antibody-producing B cells in the bursa of Fabricius, resulting in immunosuppression, which leads to vaccination failures and susceptibility to other infections (Saif, 2008). IBDV infected Chickens show suppression of both humoral and cellular immunity (Pradhan *et al*, 2014).

Traditional vaccines are mainly composed of attenuated or heat-inactivated viruses often generating many unwanted side effects. Subunit protein and peptide vaccines are generally very safe vaccines with well-defined components. However, proteins and peptides are often poorly immunogenic (Seth *et al*, 2015) and thus require the use of adjuvants to induce adequate immunity. Therefore, particulate adjuvants have been widely investigated in vaccine delivery systems (Peek *et al*, 2008).

Nanoparticles based on polymers were the most widely studied nanosystems in the field of immunization because nanomaterials are able to enhance the responses of B and T cells because of their similarity with viruses in terms of size and surface characteristics (Peleteiro *et al*., 2018). In addition, because of their long-term exposure to the immune system, these nanoparticles can produce long-term immune responses thereby, providing the possibility of reducing the number of doses required to achieve protective Abs. levels. Moreover, the nanoparticle compositions demonstrated the ability to avoid hyperactivity in the immune response and, at the same time, stimulate the production of proinflammatory cytokines (Bachmann and Jennings ,2010).

1- In particular the use of nanotechnology in vaccinology lead to “nanovaccinology” birth (Gill, 2013). Nanoparticles are used in both prophylactic and therapeutic approaches, as either a delivery system or an immunostimulant adjuvant. Therapeutic nanovaccinology is applied for treatment of cancer (Kennedy *et al*, 2011), Alzheimer (Champion *et al*, 2009), hypertension (Praetorius and Mandal, 2007) and nicotine addiction (Fifis *et al*, 2004). Nanotechnology is used in creating a new generation of effective vaccines (i.e., nanovaccines) which capable of transcending many

Ministry of Science and Technology, Department of Agricultural Research/ Iraq.
[1]Baghdad University / Veterinary Medicine collage. / Iraq.
[2]University of Technology / Department of Applied Sciences/ Iraq.
Corresponding Author: S.H. Al-Rubaee, Ministry of Science and Technology, Department of Agricultural Research/ Iraq. e-mail: dr.saja75@gmail.com

How to cite this article: S.H. Al-Rubaee, T.S. Al-Azawi and A.A. Taha (2019). Immunological Effects of a Single Dose of PLGA Nanoparticles Encapsulated Peptide in Broilers in Comparison to Traditional Vaccines against Infectious Bursal Disease. Agricultural Science Digest, 39(4): 347-352.

Source of support: Nil

Conflict of interest: None

Submitted: 20-06-2019 **Accepted:** 11-10-2019 **Published:** 09-12-2019

biological barriers in the body (Mozafari *et al*, 2009). Nanosized particles have similarly been used for vaccine construction against microbial agents (Champion *et al*, 2009). The experiment is designed to compare between broilers immunized with the prepared nanovaccine against those immunized with the traditional Gumbaro vaccine *Invivo* and investigate the physiological and immunological effects of both vaccines.

Materials and Methods

Preparation of PLGA nanoparticles loaded with peptide

PLG nanoparticles encapsulating peptide(GDQMSWSASG SLAVT) were prepared by solvent evaporation method (McCall and Sirianni,2013; Kumar, 2014), with some modification, which can be summarized: 200 mg of PLGA dissolved in 2 ml of DMSO and left at room temperature overnight, PVA was prepared by dissolving 2gr in 100 ml of diH_2O. The PLGA solution added drop wise to PVA and left overnight on stirrer then the product solution centrifugal and wash 3X and the precipitate collected and lypholized for 72hr.

Animals

Ninety eight day old (40.5 gr. Weight), unsexed broiler (Ross) chicks were brought from a local hatchery and randomly divided equally into 7 groups (14 chick) fed on an ordinary diet, starter from day 1 to 20 (22 % crud protein, 2926 K Cal / Kg) and finisher from day 21 to 42(19 % crud protein, 3109 K cal / Kg) ad libitum. The experimental animal were divided into: G1(Control) were received diH_2O by oral administration;G2- were received the traditional vaccine by oral administration;G3 were received PLGA nanoparticle by oral administration;G4 were received prepared vaccine (160 µg of peptide loaded PLGA) by oral administration;G5 were received prepared vaccine (80 µg of peptide loaded PLGA) by oral administration;G6 were received prepared vaccine (40 µg of peptide loaded PLGA) by oral administration; and G7 were received prepared vaccine (20 µg of peptide loaded PLGA) by oral administration. At day 10 of chicks' age blood was collected from wing vein randomly from 18 chicks and the serum separated for detecting antibody titration for ND and IBDV (maternal immunity) to design vaccination schedule. The exp. was carried out in private poultry farm from 3/10/2018 until 13/ 11/2018 (42 day).

Total white blood cells count, differential W.B.C. Count and H/ L ratio

Blood was collected from wing vein at the end of the experiment (42 day old) in tubes with anticoagulant (EDTA). The count was done in Veterinary laboratories/ hematology laboratory by using Orphee appliance (Switzerland).

Antibody Titer for IBD

To determine the Ab titration for IBD blood was collcted from wing vein in 10,29 and 42 days old chick. The serum was separated by using a centrifuge (800B centrifuge, China) and the determination was done according to the directive of manufactured company (Synbiotics Corpoation) (appendex1). And the below mention equation was applied:

LOG10 TITER=(1.172× LOG10Sp)+3.614
TITER=ANTILOG OF LOG10 TITER

Table 1: Vaccination schedule.

Vaccine	Chick age (day)	Route of administration
1st ND	17	Drinking water
IBDV	19	Oral administration by syringe cavage
2nd ND	27	Drinking water

Chicken Interferon Gamma (IFN-γ) by (ELISA)

Interferon Gamma (IFN-γ) was detected in the serum of broiler at the end of study, depending on the direction of manufactured company (Cloud-Clone Corp.).

Statistical analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). One-way ANOVA and least significant differences (LSD) post hoc test were performed to assess significant differences among means. ($P < 0.05$) was considered statistically significant (SAS, 2010).

Results and Discussion

Total White blood cells count, differential W.B.C count, and H/ L ratio

Fig (1A) represents the mean values of total leukocytes count among groups. The figure shows no significant ($p \geq 0.05$) differences between groups that received ordinary vaccine(G2), PLGA nanoparticles(G3), PLGA+160µg of peptide (G4), PLGA+80µg of peptide (G5), PLGA+40µg of peptide (G6) and PLGA+20µg of peptide (G7) compared with control (G1). On the other hand, broilers that received PLGA nanoparticles alone (G3) revealed significant ($p \leq 0.05$) increase in lymphocyte number with a significant ($p \leq 0.05$) decrease in hetrophils number (Fig1B) and H/ L ratio (Fig1C). The number of hetrophils and H/ L ratio showed higher significant ($p \leq 0.05$) value in group of broilers that received PLGA+160µg of peptide (G4).

Incorporation of antigens in nanoparticles can be achieved by encapsulation (physical entrapment) or by conjugation (covalent functionalization) (Chattopadhyay *et al*., 2017). Studies have demonstrated that nanoparticles could protect the native structure of antigens from proteolytic degradation and/or improve antigen delivery to antigen-presenting cells (APCs) (Pachioni-Vasconcelos *et al*., 2016). In addition, nanoparticles incorporating antigens can exert a local depot effect, ensuring prolonged antigen presentation to immune cells (Fredriksen and Grip 2011). Interestingly, nanoparticles have also shown intrinsic immunomodulatory activity (Mamo and Poland 2012). For instance, nanoparticles such as poly (lactic-co-glycolic acid) (PLGA) have been reported to induce NLRP3-associated inflammasome activation (Zhu *et al*., 2014) which subsequently activates the formation of the inflammasome complex (He *et al*., 2016). Subsequently, interleukins are produced as downstream signaling events, leading to the recruitment and/or activation of immune cells (Scharf *et al*., 2012). Taken together, these properties advocate that nanoparticles are promising antigen carriers and immune cell activators for vaccination.

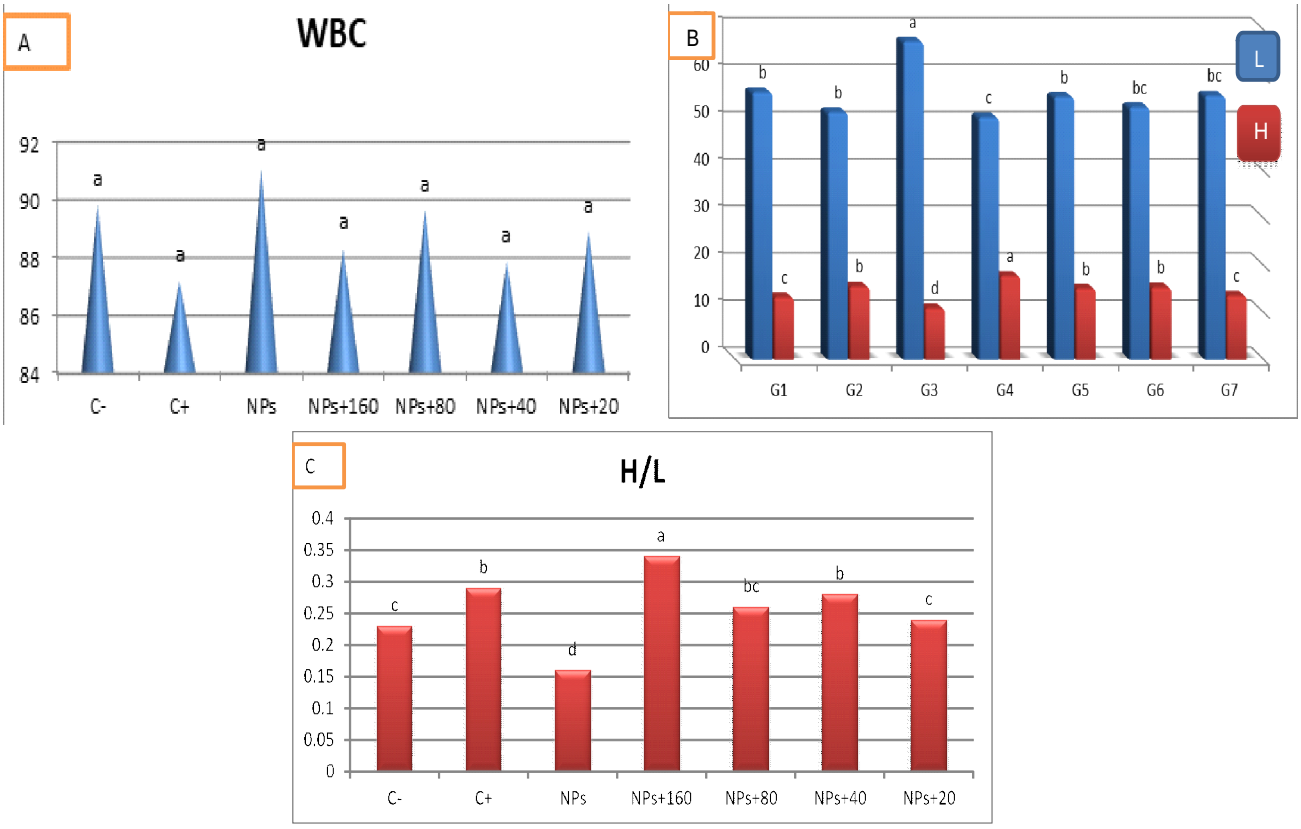


Fig 1: Effect Of PLGA Nps and PLGA loaded with different concentration of peptide on the H/ L ratio in broilers. G1=received diH_2O, G2= ordinary vaccine, G3=PLGA nanoparticle, G4= PLGA+160 µg peptide, G5=PLGA+80 µg peptide,G6=PLGA+40 µg peptide, G7=PLGA+20 µg peptide. WBCs count (A); Lymohocyte and Heterophil count (B) and H/ L ratio(C).

Also these polymers have the ability to activate cellular immune response in the host by interact and activate various toll like receptors, thus involving several innate immune system players in immune response,this increase as a result of enhancing in IL6 and IFN γ (El Naggar *et al*., 2017). The delivery of antigens to B cells via NPs has a co-stimulatory effect on Th cells, which helps to trigger specific antibody responses. The crosstalk between B cells and T cells widens the potential of utilizing NPs as vaccine adjuvants or vaccines (Du *et al*., 2017).

In our study there were no significant differences among groups and thus is in lins with the finding of Kumar (2014), who observed no considerable changes after administration of Nps to mice with endometriosis. Also Krucinska *et al*. (2017) recorded no significant changes in WBCs and thrombocytes parameters in rabbits. There were significant increase in lymphocyte count and that may be attrebuted to the increasing IFNγ concentration which play an important role in the cellular immunity and antiviral processand stimulate lymphocyte proliferation specially T- lymphocyte Gao *et al*.,2011). Saroja *et al*. (2011) and Bolhassani *et al*. (2014) showed that PLGA microspheres are rapidly taken up by M-cells and translocated toward the underlying lymphatic tissue within 1 h. For instance, the loading of Hepatitis B core antigen into PLGA NPs (300 nm) induced a stronger cellular immune response as compared with Hepatitis B core antigen alone in a mouse model.

Antibody of IBD

The available data reported in Figure (2) denote a significant ($p \leq 0.05$) increase in the Ab titration for IBD in broilers subjected to PLGA Nps (G3) at 29 and 42 days of age. Besides that, the table shows variable results of increasing Ab titer at 29 and 42 days of broilers age for other treated groups.

The humoral immune response is an essential component of protection against IBDV. In order to evaluate the immune response triggered by oral IBDV administration, Carballedaa *et al*. (2011) monitored the specific antibody levels in serum of IBDV inoculated chickens at 0, 7, 14, 21 and 35 dpi and showed that a specific anti-IBDV humoral immune response began to be detected at 7 dpi, which reached the maximum titre at 21 dpi. The level of antibodies remained high until 35 dpi. In our immunization trial, we also observed the similar antibody pattern. High levels of antibody response to PLGA nanoparticles have been previously reported by many workers (Raghuvanshi *et al*. 2002; Gutierro *et al*, 2002).

Binjawadagi *et al*. (2014) had shown that the cross protective immune response in pigs can be produced by adjuvanted poly (lactic-co-glycolic) acid nanoparticles entrapped inactivated porcine reproductive and respiratory syndrome virus vaccine.

Many researches have indicated that polymeric nanoparticles (NPs) can be used as potent adjuvants as

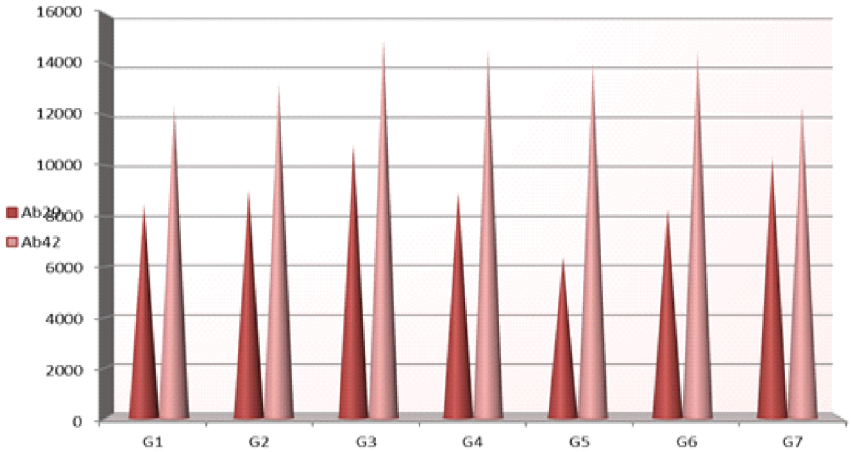


Fig 2: Effect of PLGA Nps and PLGA loaded with different concentration of peptide on IBD Antibody Titration in Serum at 10,29 and 42 days in broilers. G1=received diH_2O, G2= ordinary vaccine,G3=PLGA nanoparticle, G4= PLGA+160 µg peptide, G5=PLGA+80 µg peptide,G6=PLGA+40 µg peptide, G7=PLGA+20 µg peptide. Abs titration at 29 day ; Abs titration at 42 day

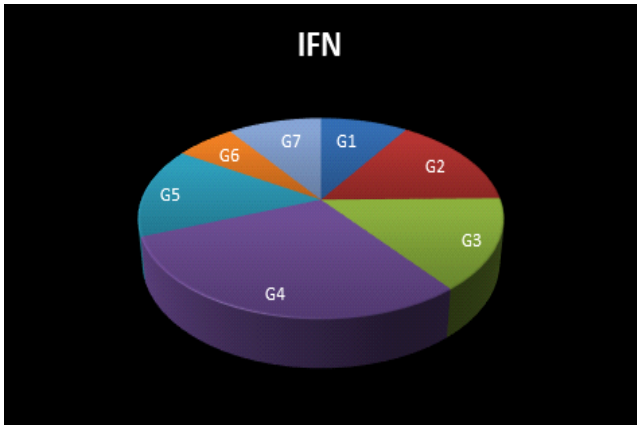


Fig 3: Effect of PLGA Nps and PLGA loaded with different concentration of peptide on level of INF-γ in serum of broilers. G1=received diH_2O, G2= ordinary vaccine, G3=PLGA nanoparticle, G4= PLGA+160 µg peptide, G5=PLGA+80 µg peptide, G6=PLGA+40 µg peptide, G7=PLGA+20 µg peptide.

part of a "nano" mucosal immune delivery system. NPs are biodegradable and biocompatible, have low toxicity and protect the antigen or DNA from damage (Zhao *et al*., 2014). Among all the polymers, polyesters based on polylactic acid, polyglycolic acid, and their copolymers, poly(lactic-co-glycolic) acids (PLGAs), have attracted the most attention and have been used as carriers for a wide range of vaccines(Hsu *et al*.,2011). Also Noh 2013) said that intranasal immunization of C57BL/6 mice resulted in antigen-specific increases in IgG, IgA and IFN-γ in sera. Ayari-Riabi(2016) mention that used of polymeric NPs as a safe adjuvant to deliver a venom fraction causing increase antibody levels in mice serum and resulted in increased immunity. Zhao *et al.* (2010) previously reported that PLGA-NPs that were prepared with a water/oil/water double emulsion-solvent evaporation method did not change the encapsulated plasmid DNA but promoted the sustained release of the plasmid DNA and induced stronger mucosal immune responses than for nonencapsulated plasmid DNA.

This indicated that delivery of vaccine antigen through PLGA nanoparticles was able to provide a sustained immunity as compared to the commercial vaccine.

Interferon gamma (INF-γ) level

From the data presented in Fig (3), it was observed that the INF-γ has highly significant ($p \leq 0.05$) mean in serum of broilers received PLGA+160µg (G4) at day 42 of age as compared with control and other treated groups.

Interferons (IFNs) are important members of host innate arm of immunity to prevent virus infection by their antiviral effect (El-Naggar *et al*., 2017). IFN-γ produced by CD4+ T helper cell type 1 (Th1) lymphocytes, CD8+ cytotoxic lymphocytes and NK cells (Schroder *et al*., 2004). However, there is now evidence that other cells, such as B cells, NKT cells and professional antigen-presenting cells (APCs) secrete IFN-γ (Harris *et al*.,2000). IFN-γ production by professional APCs (monocyte/macrophage, dendritic cells (DCs) acting locally may be important in cell self-activation and activation of nearby cells (Frucht *et al*., 2001). IFN-γ secretion by NK cells and possibly professional APCs is likely to be important in early host defense against infection, whereas T lymphocytes become the major source of IFN-γ in the adaptive immune response (Sen, 2001).

Noh (2013) said that intranasal immunization of C57BL/6 mice resulted in increases in IFN-γ in sera. Shakya and Nandakumar(2012) show that the nanoparticles and polymers vaccine adjuvant could enhance the efficacy of avian mucosal vaccination against infectious diseases by increasing the expression of IFN γ and IL6 genes to provide insights into the role of innate immune response in protection against infectious disease in chicken. Also Hu *et al*. (2012) observed increase the phagocytic activity as a result of enhancing in IL-6 and IFN-γ. Jahan *et al*. (2018) observed that the highest amount of IFN-γ was secreted after DC stimulation with OV-NPs in compared with the stimulation of free OV.

Conclusion

Infectious bursal disease in poultry is an economic disease causes economic losses due to the weight loss and secondary infection associated with the disease. The results of the experiment indicate that the use of nanoparticles in the preperation of a vaccine against IBD cause a significant increase in the number of lymphocytes and significant decrease in heterophil, especially in G3, which orally administered with PLGA NPs and recorded this group the lowest level in the proportion of H \ L ratio . For the level of antibodies, it is noted that the highest values were recorded in G3, G4, G5 and G6 compared to the other groups on the 29th and 42 of the experiment. INF-γ recorded the highest level at the end of the experiment in G4. This shows that the use of PLGA NPs as adjuvante act as stimulator and stimulate immunity in both cellular and humoral arms in poultry better than traditional vaccine.

References

Ayari-Riabi, S. (2016). Venom conjugated polylactide applied as a biocompatible material for passive and active immunotherapy against scorpion envenomation. Vaccine. 34: 1810–1815.

Bachmann MF, Jennings GT. Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. Nat Rev Immunol (2010) 10(11): 787–96.

Binjawadagi, B., Dwivedi, V., Manickam, C., Ouyang, K., Wu, Y., Lee, L.J., Torrelles, J.B. and Renukaradhya, G.J. (2014). International Journal of Nanomedicine. 9: 679–694.

Bolhassani,A.; Javanzad,S.; Saleh,T.; Hashemi,M.; Aghasadeghi,M.R.; and Sadat,S.M.(2014). Polymeric nanoparticles Potent vectors for vaccine delivery targeting cancer and infectious diseases. Human Vaccines & Immunotherapeutics 10(2): 321–332.

Carballedaa,J.M.; Zotha,S.C.; Gomeza,E.; Gravisacoa,M.J.; Berinsteina, A.(2011). Activation of the immune response against Infectious Bursal Disease Virus after intramuscular inoculation of an intermediate strain. Immunobiology. 216: 1028– 1033.

Champion, C.I., Kickhoefer, V.A., Liu, G., Moniz, R.J., Freed, A.S., Bergmann, L.L., et al. (2009). ''A vault nanoparticle vaccine induces protective mucosalimmunity'', PLoS ONE. 4(4): Art. no. e5409.

Chattopadhyay,S.; Chen,J.Y.; Chen, H.W.; Hu,C.M.(2017). Nanoparticle Vaccines Adopting Virus-like Features for Enhanced Immune Potentiation. Nanotheranostics; 1(3): 244-260.

Du,J.; Zhang,Y.S.; Hobson,D.; Hydbring,P. (2017): Nanoparticles for immune system targeting. Drug Discov Today, http://dx.doi.org/10.1016/j.drudis.2017.03.013.

El Naggar HM, Madkour MS, Hussein HA (2017) Preparation of mucosal nanoparticles and polymer-based inactivated vaccine for Newcastle disease and H9N2 AI viruses, Veterinary World. 10(2): 187-193.

Fifis, T., Mottram, P., Bogdanoska, V., Hanley, J. and Plebanski, M. (2004). ''Short peptide sequences containing MHC class I and/or class II epitopes linked to nano-beads induce strong immunity and inhibition of growth of antigen-specific tumour challenge in mice''. Vaccine, 23(2): pp. 258–266.

Fredriksen BN, Grip J. (2012). PLGA/PLA micro- and nanoparticle formulationsserve as antigen depots and induce elevated humoral responses after immunization of Atlantic salmon (Salmo salar L.). Vaccine. 30: 656–67.

Frucht, D. M., Fukao, T., Bogdan, C., Schindler, H., O'Shea, J. J., Koyasu, S. (2001) IFN-gamma production by antigen-presenting cells: mechanisms emerge. Trends Immunol. 22: 556–560.

Gao,L.;Qi,X;Li,K.;Gao,H.;Gao,Y.;Qin,L.;Wang,Y.;andWang,X.(2011). Development of a tailored vaccine against challenge with very virulent infectious bursal disease virus of chickens using reverse genetics. Vaccine. 29: 5550– 5557.

Gill, P. (2013): Nanocarriers, nanovaccines, and nanobacteria as nanobiotechnological concerns in modern vaccines. Scientia Iranica F. 20(3): 1003–1013.

Gutierro, I., Hernandez, R.M, Igartua, M., Gascon, A.R. and Pedraz, J.L. 2002. Size dependent immune response after subcutaneous, oral and intranasal administration of BSA loaded nanospheres. Vaccine. 21: 67-77.

Harris, D. P., Haynes, L., Sayles, P. C., Duso, D. K., Eaton, S. M., Lepak, N. M., Johnson, L. L., Swain, S. L., Lund, F. E. (2000). Reciprocal regulation of polarized cytokine production by effector B and T cells. Nat. Immunol. 1: 475–482.

He Y, Hara H, Núñez G. Mechanism and regulation of NLRP3 inflammasome activation. Trends Biochem Sci. (2016) 41:1012–21.

Hsu SH, Chan SH, Chiang CM, Chen CC, Jiang CF. (2011): Peripheral nerve regeneration using a microporous polylactic acid asymmetric conduit in a rabbit long-gap sciatic nerve transection model. Biomaterials. 32(15):3764–3775/

Hu, C. H., L. Y. Gu, Z. S. Luan, J. Song, and K. Zhu. (2012). Effects of montmorillonite-zinc oxide hybrid on performance, diarrhea, intestinal permeability and morphology of weanling pigs. Anim. Feed Sci. Technol. 177:108-115.

Jahan, S.T.; Sadat, S.M.; Haddadi, A. (2018): Design and immunological evaluation of anti-CD205-tailored PLGA-based nanoparticulate cancer vaccine. International Journal of Nanomedicine. 13: 367–386.

Kennedy, L.C., Bickford, L.R., Lewinski, N.A., Coughlin, A.J., Hu, Y., Day, E.S.,West, J.L. and Drezek, R.A. (2011). "A new era for cancer treatment: goldnanoparticle-mediated thermal therapies". Small. 7(2): pp. 169–183

Krucinska,I; Zywicka, B; Komisarczyk,A.; Szymonowicz,M.; Kowalska,S.; Zaczynska,E.; Struszczyk,M.; Czarny,A.; Jadczyk,P.; Uminska-Wasiluk ,B.; Rybak,Z.; and Kowalczuk,M.(2017): Biological Properties of Low-Toxicity PLGA and PLGA/PHB Fibrous Nanocomposite Implants for Osseous Tissue Regeneration. Part I: Evaluation of Potential Biotoxicity. Molecules, 22: 2092.

Kumar, V (2014). Chitosan Coated Plg Nanoparticles as Delivery System for Infectious Bursal Disease Viral Antigens. PhD Thesis, Deemed University Indian Veterinary Research Institute, Izatnagar - 243 122 (U.P.), India.

Mamo T, Poland GA. (2012): Nanovaccinology: the next generation of vaccines meets 21st century materials science and engineering. Vaccine. 30: 6609–11.

McCall,R. and Sirianni,R.(2013): PLGA Nanoparticles Formed by Single- or Double-emulsion with Vitamin ETPGS. Journal of Visualized Experiments. 82:1-8.

Mozafari, M.R., Pardakhty, A., Azarmi, S., Jazayeri, J.A., Nokhodchi, A. and Omri, A. (2009). "Role of nanocarrier systems in cancer nanotherapy", J. Liposome Res. 19(4). pp. 310–321.

Noh, Y.W. (2013) Polymer nanomicelles for efficient mucus delivery and antigen-specific high mucosal immunity. Angew. Chem. Int. Ed. Engl. 52: 7684–7689.

Pachioni-Vasconcelos Jde A, Lopes AM, Apolinario AC, Valenzuela-Oses JK, Costa JS, Nascimento Lde O, (2016). Nanostructures for protein drug deliver. Biomater Sci. 4: 205–18.

Peek, L.J.; Middaugh,C.R.; and Berkland,C.(2008).Nanotfchnologyin vaccine delivary. Advanced drug delivary review. 60: 915-928.

Peleteiro M.; Presas E.; González- Aramundiz JV. Sánchez-Correa B.; Simón-Vázquez R.; Csaba N.; Alonso MJ. and González- Fernández Á (2018). Polymeric Nanocapsules

for Vaccine Delivery: Influence of the Polymeric Shell on the Interaction with the Immune System. Front. Immunol. 9:791.

Pradhan,S.N.; Prince,P.R.; Madhumathi,J.; Arunkumar,C.; Roy,P.; Narayanan,R.B.;and Usha Antony(2014): DNA vaccination with VP2 gene fragment confers protection against Infectious Bursal Disease Virus in Chickens. Vet. Microbiol. VETMIC-6521; No. of Pages 10

Praetorius, N.P. and Mandal, T.K. (2007). "Engineered nanoparticles in cancer therapy", Recent Pat. Drug Deliv. Formul. 1(1): pp. 37–51.

Raghuvansi, R.S., Goyal, S., Singh O. and Panda, A.K. 2002. Formulation and characterization of immunoreactive tetanus toxoid biodegradable polymer particles. Drug deliv. 3: 113-120.

Saif, Y. M. and; Eterradossi,N(2008): Infectious Bursal Disease.In "Diseases of Poultry". 12th Edition, Ch.7, pp185-208, Blackwell Publishing,

Saroja Ch, Lakshmi PK, Bhaskaran S. (2011): Recent trends in vaccine delivery systems: A review. Int J Pharm Investig 1: 64-74.

SAS.2010.SAS/STAT Users Guide for Personal Computer. Release 9.13.SAS Institute, Inc., Cary, N.C., USA.

Scharf B, Clement CC, Wu XX, Morozova K, Zanolini D, Follenzi A, et al. Annexin A2 binds to endosomes following organelle destabilization by particulate wear debris. Nat Commun. (2012) 3:755.

Sen, G. C. (2001) Viruses and interferons. Annu. Rev. Microbiol. 55: 255–281.

Seth,A.; Ritchie,F.K.; Wibowo,N.; Lua,L.H.; Middelberg,A.P.J.(2015). Non-Carrier Nanoparticles Adjuvant Modular Protein Vaccine in a Particle-Dependent Manner. Research Article, journal.pone:1-16.

Shakya, A.K. and Nandakumar, K.S (2012). Applications of Polymeric Adjuvants in Studying Autoimmune Responses and Vaccination against Infectious Diseases. Medical Inflammation Research, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden.

Thaiss, C.A. (2016). The microbiome and innate immunity. Nature 535: 65–74.

Zhao K, Li GX, Jin YY (2010). Preparation and immunological effec-tiveness of a Swine influenza DNA vaccine encapsulated in PLGA microspheres. J Microencapsul. 27(2):178–186.

Zhao,L.; Seth,A., Wibowo,N.; Zhao,C.; Mitter,N.; Yu,C.;and Middelberg, A. (2014). Nanoparticle vaccines. Vaccine 32: 327– 337.

Zhu M,Wang R, Nie G. Applications of nanomaterials as vaccine adjuvants. Hum Vaccin Immunother. (2014)10: 2761–74.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/ licenses/by/4.0/.© The Author(s) 2019

ISSN: 0976-0547 (Online), 0253-150X (Print), Naas Rating: 4.21