P-ISSN: 2304-3075; E-ISSN: 2305-4360

International Journal of Veterinary Science

www.ijvets.com; editor@ijvets.com

Research Article

Efficacy of Preliminary Prepared Mucosal Vaccine of Infectious Coryza in Chicken

Sayed RH[1], Shasha FA[1], Noura M Khalaf[2], Sahar M Saber[1] and Lamees A El- Tantawy[2]

[1]Central Laboratory for Evaluation of Veterinary Biologics, Abbasia, Cairo, Egypt
[2]Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt
***Corresponding author:** rafikhamed1010@hotmail.com

Article History: Received: May 18, 2019 Revised: May 28, 2019 Accepted: May 29, 2019

ABSTRACT

Infectious Coryza (IC) is an acute respiratory disease of chicken that caused by *Avibacterium paragallinarum* types A, B and C. Protection of chicken flocks against IC using inactivated vaccine may not totally protect chicks because of lake of vascularity in the infraorbital sinus that permit low amount of antibodies against AP to reach this region. So, in this work poly D, L lactide-co-glycolic acid (PLGA) nano emulsion was used for preparation of mucosal IC vaccine. The immunizing purified hemagglutinin antigen of the three serotypes was prepared and emulsified with PLGA nano emulsion. About 270 SPF chicks (5weeks Ago) were divided into three groups. The first group was dropped intranasally with mucosal IC vaccine; second group was injected with traditional oil adjuvant IC inactivated vaccine while the third group was left as unvaccinated control group. Serum sample were collected weekly and intranasal challenge test was applied using virulent *AP* at 3 weeks post vaccination. ELISA antibody titers for mucosal and inactivated vaccine reached to 3272 and 1324 respectively at 7 weeks post vaccination. The protection rates of challenge test were 95% & 87% for mucosal and inactivated vaccine respectively.

Key words: *Avibacterium paragallinarum*, Nano emulsion, Chicken

INTRODUCTION

Haemophilus paragallinarum is known to be the causative agent of infectious coryza (I.C) (Delaplane *et al*., 1934; Elliot and Lewis, 1934) which has been described in the early literature as roup, contagious or infectious catarrh and uncomplicated coryza (Yamamoto, 1991). It is observed that there is a unique group within the bacterial family *Pasteurellaceae* associated with avian hosts and these bacteria are rarely isolated from any other host species, this causative agent is known as *Avibacterium paragallinarum* (Blackall *et al*., 2005).

The economic impact of the disease attributes to increase culling rate in meat chicken and 10-40% reduction in egg production in laying and breeding hens causing severe economic losses in egg industry in many parts of the world (Yamamoto, 1984; Blackall *et al*., 1990; Yamamoto, 1991 and Yamaguchi *et al*., 1993),

In recent years, Nano vaccine is a novel approach to the methodology of vaccination. Nanomaterials are delivered in the form of microspheres, nanobeads or micro-nano projections. Painless, effective and safe needle-free routes such as intranasal or oral route or patches of micro projections to the skin are some of the approaches which are in the experimental stage at present but may have a great future ahead in nano vaccination (Nandedkar, 2009).

PLGA reported as one of the most widely studied polymers of interest in the vaccine field. Since PLGA polymers can offer long-term release of their contents in a recurring, pulsatile manner, the primary focus of past studies has been in using them to replace the multiple immune boosting administrations typically required to induce protective immunity, the using nano microparticles prepared from biodegradable and bio-compatible polymers to induce both humoral and cellular immune responses (Scheerlinck *et al.,* 2007).

PLGA polymers can potentially deliver antigens or adjuvants to a desired location at predetermined rates and durations effectively regulating the immune response over a period of time (Zhao and Leong, 1996).

Recent studies show that co-encapsulation of both antigen and adjuvant in nanoparticulate carriers have synergistic effects in augmenting immunity by targeting both antigen and adjuvant to the endosomal compartment of APCs (Kasturi *et al*., 2011).

Cite This Article as: Sayed RH, FA Shasha, NM Khalaf, SM Saber and LA El- Tantawy, 2019. Efficacy of preliminary prepared mucosal vaccine of infectious coryza in chicken. Inter J Vet Sci, 8(3): 182-186. www.ijvets.com (©2019 IJVS. All rights reserved)

So, the aim of this work was to investigate the effect of mucosal vaccine (nano vaccine), namely PLGA entrapping HA protein of *Avibacterium paragallinarum*, as an adjuvating immuno stimulant agent with nasal dropping administration to induce both humoral and cellular immune responses in the low lake vascularity region.

MATERIALS AND METHODS

***Avibacterium paragallinarum* bacterial strains:** AP strains serotypes A, B &C were obtained from the Anareobic Bacterial Vaccine Department, Vet. Ser. Vac. Res. Institute. Abbassia, Cairo.

Molecular identification using multiplex PCR (Patil *et al.*, 2017): The DNA was extracted from the three serotype strains by using easy pure bacterial genomic DNA extraction kit *(Trans, China)*. Briefly, the PCR reactions were carried out for a total volume of 20 µl containing 10 micro 1X PCR master mix solution (iNtRON), 2 µl template DNA, 1µl forward primer, 1µl for each reverse primer and 6 µl distilled water. The primers sequence was mentioned in Table 1. The amplification step was as follows 98ºC for 1 min, 30 cycles for 98ºC for 10 sec, 56ºC for 10 sec and 72º C for 2 min; and final extension 72ºC for 7 mins. The PCR product sizes were determined using agarose gel electrophoresis 1%.

Preparation of *Avibacterium paragallinarum* hemagglutinin antigen (HA) (Yamaguchi *et al.,* 1990): The three serotypes of *Avibacterium paragallinarum* strains were grown in Kato's broth medium containing 1% sterile chicken serum and 2.5 µg/ ml NADH at 37ºC in 10% CO_2 for 18 hours. The bacterial cells were collected by centrifugation at 8000 rpm for 20 minutes and suspended in 0.02 M phosphate buffered saline (pH 7.2). The cells were collected and washed three times in PBS (pH 0.7), then re-suspended in 0.5 M KSCN/0.425M NaCl pH 6.3 and adjusted to an optical density of 1.6 at 650 nm. The suspensions were put in 4ºC for 2 hrs with agitation then sonicated (30sec, 60% pulsed output, power output 5). The sonicated antigens were washed three times in PBS, then re-suspended in PBS with 0.01% (w/v) thimerosal and adjusted to optical density of 1.6 at 650 nm.

Protein precipitation (Lovrien and Matulis, 2001): Protein preceptiation was performed through ammonium sulfate saturation technique. The protein concentration was determined through spectrophotometer.

Preparation of mucosal nano vaccine

Preparation of PLGA nanospheres (Zaman *et al.*, 2013): PLGA nano vaccine was prepared using a W/O/W emulsion and solvent technique. One mg from HA antigen of the three serotypes was dissolved in 150 µl water for injection. The antigen solutions were mixed and emulsified in PLGA (sigma) solution (200mg PLGA in 600µl Dichloromethane (sigma) by probe sonication for 80 sec. The resulting W/O emulsion was then emulsified in 8 ml polyvinyl alcohol (PVA) solution (5% W/V). This secondary emulsion was then added to 32ml of 5% W/V PVA solution and stirred for 3 hrs. to evaporate the organic solvent. Nanospheres were collected by centrifugation (15000rpm for 45mins) and washed twice with distalled water.

Characterization of PLGA nanospheres: Determination of surface properties of PLGA nanospheres was carried out using Scanning Electron Microscopy (SEM). The SEM photographs were taken at different magnification levels with a size bar imprinted on the micrographs.

Determination of (HA) encapsulation efficiency (loading level) in PLGA nanospheres: The dose of HA protein entrapped in, was calculated based on actual entrapment of the antigen in the nanospheres. The determination was carried out by Qubit fluorometer. Ten µl samples of PLGA nanospheres was transferred in 1.5 ml Eppendorf tube. One ml of 0.2N NaOH 1% sodium dodecyl sulfate (SDS) solution was added to vials. The vials were placed on a constant shaker at room temperature. The PLGA- HA nanospheres were allowed to hydrolyze overnight, and then the hydrolyzed sample was subjected to protein estimation.

Determination of in vitro release of HA proteins from PLGA nanospheres along with checking of their stability: Ten samples of PLGA-HA nanospheres suspension of 110 µl each were added to 2 ml of PBS (pH 7.4) and incubated at 25 and 41ºC on a constant shaking mixer. One volume was withdrawn at each time-point of the *in vitro* estimation, at days 1, 3, 7, 14, 21, 28, 32, 36, 40 and 49. The PLGA- HA nanosphere suspensions were centrifuged at 5000 rpm for 10 min and the supernatant was collected. The supernatant was used for protein estimation at various time-points in the release kinetics, and percent cumulative release of the antigen versus time was plotted.

Experimental design: A total of 270, five week old SPF chickens were divided into 3 groups. The first group (90 chickens) was vaccinated with the mucosal nano vaccine (one drop intranasal) and second group (90chickens) was vaccinated with imported oil adjuvant vaccine (0.5ml S/C), while the third group (90 chickens) used as control unvaccinated chickens.

Blood samples were collected before immunization and at one week interval post immunization to evaluate the developed immune response to the vaccine. Sera were separated and collected separately in sterile tubes and kept at -20°C for testing by ELISA.

Table 1: Primer sequence of *AP* serotypes strains

Primer	Sequence (5'-3')	Product size
A, B, C Forward primer	GGCTCACAGCTTTATGCAACGAA	
A Reverse primer	CGCGGGATTGTTGATTTTGTT	800 bp
B Reverse primer	GGTGAATTTCACCACACCAC	1100 bp
C Reverse primer	TAATTTTCTTATTCCCAGCATCAATACCAT	1600 bp

Protective efficacy was determined according to (Wambura, 2010). Fifty chickens from each vaccinated groups were challenged intranasal with 0.2 mL (10^6CFU) viable local *Avibacterium paragallinarum* cells for each serotype. After challenge, the chickens were examined daily for clinical signs of infectious coryza for fifteen days.

Re-isolation of bacterial strains after challenge test according to (Wambura, 2010): The chickens in all groups were euthanized at 2 week post challenge. Infraorbital sinus swabs were taken to determine the evidence of *Avibacterium paragallinarum* shedding using multiplex PCR.

Serological tests: Enzyme-Linked immunosorbent assay (ELISA) was carried out as outlined by (Myers, 1995)

RESULTS

Fig 1 Illustrated results of multiplex PCR for the three serotypes of AP, specific bands were shown at different molecular weights.

Fig 2 showed PLGA nanospheres antigen as examined by transmission electron microscope. PLGA appeared as layered spherical and semi-spherical particles with average size range from 400 nm to 950 nm.

The intermittent tracing of released protein in supernatant could give a clear picture about the kinetics of antigen release from PLGA nanospheres at 25ºC and 40ºC increased gradually till reached 5.21 and 7.4 respectively as shown in Table 2.

ELISA test: In mucosal vaccine the ELISA antibody titer against *Avibacterium paragallinarum* reached 3272 at 7th week post vaccination while in inactivated vaccine reached 1324 at the same time as shown in Table 3.

Challenge test: The protection rate of mucosal vaccine was 95% at 3rd week post vaccination while it was 87% in inactivated vaccine as shown in Table 4.

Fig. 1: molecular identification of *Avibacterium paragallinarum A, B and C serotypes;* Lane (M): 3000- 100 bp DNA ladder (Fermentas). Lane (1) Showed band at 800 bp specific for *Avibacterium paragallinarum A* serotype. Lane (2) Showed band at 1100 bp specific for *Avibacterium paragallinarum B* serotype. Lane (3) Showed band at 1600 bp specific for *Avibacterium paragallinarum C* serotype. PLGA nanospheres character.

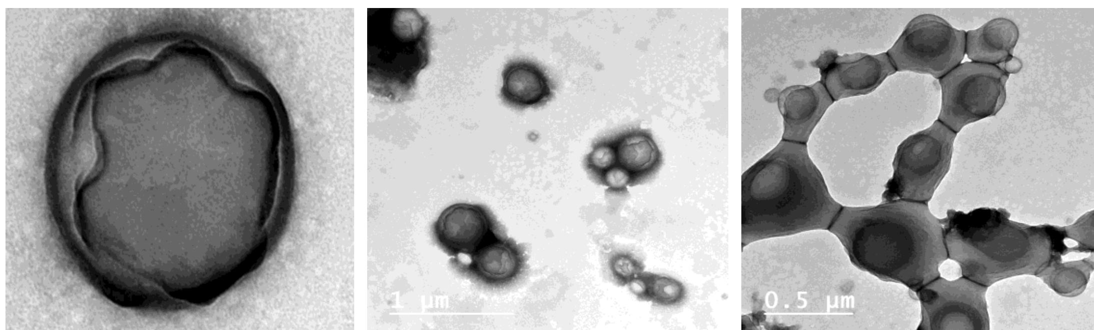

Fig. 2: Characterization of PLGA nanospheres: (HA) encapsulation efficiency (loading level) in PLGA nanospheres: Protein concentration of hemagglutinin antigen was 0.899 µg/ 10 µl hemagglutinin antigen. *in vitro* release of HA proteins from PLGA nanospheres along with checking of their stability.

Table 2: In vitro release of hemagglutinin antigen from PLGA nano spheres at 25ºC and 40ºC.

Temperature	Protein concentration (µg) / 110 µl estimated in different times (in days) for PLGA nanoparticles								
	1	**4**	7	14	21	28	35	42	49
at 25 °C	0.001	0.002	0.06	0.89	1.34	2.22	3.56	4.31	5.21
at 40 °C	0.002	0.02	1.2	2.1	3.4	5.9	6.9	7	7.4

Table 3: ELISA antibody titers in vaccinated chicken groups.

Weeks post vaccination	Inactivated vaccine	Mucosal vaccine	Control group
1st	419	567	258
2nd	561	1782	301
3th	750	2398	289
4th	982	2780	322
5th	1009	3012	278
6th	1298	3210	256
7th	1324	3272	309

ELISA antibody titer was 328 in all chicken before vaccination.

Table 4: The protective efficacy of the mucosal vaccine and Inactivated vaccine using challenge test 3 weeks post vaccination.

Vaccinated group	No of challenged birds	Daily observation for one week post challenge								Infection %	Protection %
		1st	2nd	3rd	4th	5th	6th	7th	Total		
Mucosal vaccine	60	0	1	1	1	0	0	0	3	5%	95%
Inactivated vaccine	60	0	2	3	2	1	0	0	8	13%	87%
Control	60	0	10	9	8	7	3	0	37	62%	----

Table 5: The evidence of *Avibacterium paragallinarum* shedding 2 weeks post challenge using multiplex PCR.

Vaccinated group	No. of swabs	Positive AP samples used Multiplex PCR 2 week post challenge			Total infected samples*	Shedding %
		Serotype A*	Serotype B*	Serotype C*		
Mucosal vaccine	60	2	4	1	7	3.9%
Inactivated vaccine	60	10	15	7	32	17.8%
Control	60	50	51	45	146	81.1%

Shedding determination: Two weeks post challenge samples from infraorbital sinuses were examined by *multiplex PCR* for determining the evidence of *Ap* shedding. The shedding percentage at mucosal and inactivated vaccine was 3.9% and 17.8% respectively while in the control group it was 81.1% as shown in Table 5.

DISCUSSION

Infectious coryza is an acute respiratory disease of chickens caused by *Avibacterium paragallinarum.* The greatest economic losses associated with infectious coryza results from poor growth performance in growing birds and marked reduction (10-40%) in egg production in layers (Blackall and Matsumoto, 2003). PLGA Nanosphere formulations have been considered as efficient adjuvants in the recent years. Intranasal administration of antigens is used to induce potent mucosal, humoral and cellular immune responses. Combinations of the above mentioned approaches are predicted to enhance immunoregulatory responses. In this study, the PLGA nanosphere formulations containing HA antigens of *Aviabacterium paragalinarium* A, B and C serotypes were prepared and characterized.

The size of PLGA Nanosphere is 400 nm to 950 nm and it plays an important role in immunoadjuvant properties of the particles. Due to this fact, increasing of the *PVA* concentration might be leads to decrease size of nanospheres. The nano-sized particles are more potent for inducing mucosal immune responses.

This study showed that nanoparticles vaccine adjuvant can enhance the efficacy of avian mucosal vaccination against Infectious Coryza. It could be demonstrated that individual intranasal delivery of adjuvanted formulations containing nanoparticles based adjuvant technologies were able to improve the immune response and protection of infectious Coryza challenge.

The use of bio degradable polymeric nanoparticles (PLGA) with entrapped antigen such as protein, peptides or DNA represents an exciting approach for controlling the release of vaccine antigens and optimizing the desired immune response via selective targeting of the antigen to Ag-presenting cells (Akagi *et al*., 2012) PLGA encapsulated immunogens are slowly and continuously released resulting in repeated stimulation of lymphocytes including antigen presenting cells (Newman *et al*., 2012).

Inactivated vaccines are preferable than live vaccines due to a possible genetic transmutation of live strains of bacterium into more pathogenic serovars (Deshmukh *et al*., 2015). Also (Chukiatsiri *et al*., 2009) stated that a new produced vaccine for infectious coryza should contain more than one strain of *Av. Paragallinarum* and local strains of *Av. Paragallinarum*.

Protection level in the challenged birds were evaluated based on the exhibited clinical signs of infectious coryza and bacterial isolation of *Av. Paragallinarum* strains, concerning the results of challenge test as shown in Table 4. It was cleared that chickens in immunized groups were protected in relative to unimmunized control group. The group vaccinated with mucosal vaccine was the most protected for chicken and provided the highest protection percentage as it was 95 %, while it was 87% in group vaccinated with inactivated vaccine as shown in Table 4. These results agree with (Deville *et al*., 2012) who used nanoparticles adjuvant intra nasal vaccine against IB virus, intranasal vaccine was able to trigger a significantly stronger humoral immune response and by challenge conferred a significantly enhanced protection. Also, these results agree with (Heba El Naggar *et al*., 2017) who used nanoparticles mucosal vaccine against ND and confirmed that mucosal vaccine induce superior protection against viruses that infect mucosal surfaces. Also (Abd El-Bakey, 2018) confirmed the use of PLGA nanoparticles adjuvant for enterotoxaemia bloat vaccine of *Clostridium perfringens* type A as it induced prolonged immunity compared with gel vaccine.

The dynamics of humoral immune response were analyzed in the two immunized groups, using one serological test (ELISA), for determination of antibody titer, prevaccination, 1st, 2nd week, 3rd, 4th, 5th, 6th and 7th weeks post vaccination for mucosal vaccine and inactivated vaccine as well as control group.

The antibody levels estimated by ELISA continued high with higher significant differences among two vaccinated groups. This finding of ELISA supported that of challenge test in mucosal and Inactivated vaccine which reached 3272,1324 at 7 weeks post vaccination with mucosal and Inactivated vaccines as shown in Table 3 respectively.

By multiplex PCR as shown in Table 5 the evidence of *AV. Paragallinarum* shedding at 2 weeks post challenge in mucosal vaccine was 2 infected samples for

serotype A,4 infected samples for serotype B, 1 infected samples for serotype C, with total infected samples 7, while in Inactivated vaccine, shedding of infected samples was 10 samples for serotype A, 15 samples for serotype B, 7 samples for serotype C with total infected samples 32.

So, it could be concluded from results of this study that nano PLGA vaccine was better immunologically and induced high protection rate reached 95% compared with Inactivated vaccine.

REFERENCES

Abd El-Bakey A, 2018. Immunological studies of *C. perfringens* type A using nanotechnology. PhD of microbiology bacteriology, immunology and mycology. Faculty of Veterinary Medicine Cairo University.

Akagi T, Baba M and Akashi M, 2012. Biodegradable Nanoparticles as vaccine. Adv Polym Sci, 247: 31-64.

Blackall PJ, Eaves LE and Aus G, 1990. Serotyping of *Haemophilus paragallinarum* by the Page scheme: comparison of the use of agglutination and hemagglutination-inhibition tests. Avian Dis, 34: 643-645

Blackall PJ and Matsumoto M, 2003. Infectious coryza. In YM Saif, HJ Barnes, JR Glisson, AM Fadly, LR McDougald and DA Swayne (Eds), Diseases of poultry, 11th edn (pp: 691-703). Ames. Iowa: Iowa State University Press.

Blackall PJ, Christensen H Beckenham T, Blackall LL and Bisgaard M, 2005. Reclassification of Pasteurella gallinarum, Haemophilus paragallinarum, Pasteurella avium and Pasteurella volantium as Avibacterium gallinarum gen. nov., comb. nov., Avibacterium paragallinarum comb. nov., Avibacterium avium comb. nov. and Avibacterium volantium comb. nov. Int J Syst Evol Microbial, 55: 353-362.

Chukiatsiri K, Sasipreeyajan J, Neramitmansuk W and Chansiripornchai, 2009. Efficacy of autogenous inactivated vaccine of Avibacterium paragalllinarum. Avian Dis, 533: 382-6.

Delaplane JP, Erwin LE and Stuart HO, 1934. A hemophilic bacillus as the cause of an infectious rhinitis. Rhode Island State College Bulletin, 244: 1-12.

Deshmukh SBH, Sodhi S and Brar RS, 2015. An update on avian infectious coryza: It is re-emerging trends n epidemiology, etiologic characterization, diagnostics, therapeutic and prophylactic advancements. J Dairy Vet Anim Res, 2: 00037.

Deville Sebastien, Juliette Ben Arous, Francois Bertrand, Vladimir Borisov and Laurent D, 2012. Efficacy of intranasal and spray delivery of adjuvanted live vaccine against infectious bronchitis virus in experimentally infected poultry. Procedia Vaccinol, 6: 85-92.

Eliot CP and Lewis MR, 1934. A haemophilic bacterium as a cause of infectious coryza in the fowl. J Amer Vet Med Ass, 84: 878-888.

Heba M El-Naggar, Mohamed SM and Hussein AH, 2017. Preparation of mucosal nanoparticles and polymer-based inactivated vaccine for Newcastle disease and H9N2 AI viruses. Vet World, EISSN: 2231-0916.

Kasturi SP, Skountzou I and Albrecht RA, 2011. Programming the magnitude and persistence of antibody responses with innate immunity. Nature, 470(7335): 543-7.

Lovrien RE and Matulis D, 2001. Selective precipitation of proteins. Curr.Protoc. Protein Sci May; chapter 4: unit 4.5.

Myers RL, 1995. Immunology. A Laboratory Manual 2nd ed. WMC Brown publishers, USA.

Nandedkar TD Chitnis S and Vavia P, 2009. Application of engineered peptide nanomaterial in reproductive health and its safety; ICONN held at Melbourne, Australia.

Newman KD, Elamanchili P, Kwon GS and Samuel J 2012. Uptake of poly) D, Llactic-co-glycolic acid) microspheres by antigen- presenting cells in vivo. J Biomed, Mater Res, 603: 480-486.

Patil VV, Mishra D and Mane DV, 2017. 16S ribosomal RNA sequencing and molecular serotyping of *Avibacterium paragallinarum* isolated from Indian field conditions, Vet World, 10(8): 1004-1007.

Scheerlinck JP, Gloster S, Gamvrellis A, Mottram PL and Plebanski M, 2006. Systemic immune responses in sheep, induced by a novel nano-bead adjuvant; Vaccine 24: 1124-1131.

Wambura PN, 2010. Preparation and use of autogenous vaccine from Avibacterium paragallinarum (strain Tan 1-05) in layer chickens. Trop Anim Health Prod, pp: 42-483.

Yamaguchi T, Kobayashi M, Masaki S and Iritani Y, 1993. Isolation and characterization of a Haemophilus paragallinarum mutant that lacks a hemagglutinating antigen. Avian Dis, 37: 970-976.

Yamaguchi T, Blackall PJ, Takigami S, Iritani Y and Hayashi Y, 1990. Pathogenicity and serovar- specific Haemagglutinating antigens of Haemophilus paragallinarum serovar B strains. Avian Dis, 34: 964-968.

Yamamoto R, 1991. Infectious coryza. In: Diseases of Poultry, 9th Ed. Hofstad, MS, Barnes, HJ, Calnek, BW, Reid WM and Yoder ER Jr (Eds). Iowa State University Press pp: 180-195.

Yamamoto R, 1984. Infectious coryza in: Disease of Poultry, 8th Ed. Iowa State University Press, Iowa pp: 178-186.

Zaman M, Chandrudu S and Toth I, 2013. Strategies for intranasal delivery of vaccines. Drug Deliv Transl Res, 3: 100-109.

Zhao Z and Leong KW, 1996. Controlled delivery of antigens and adjuvants in vaccine development. J Pharm Sci, Dec, 85(12): 1261-70.