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Comparative Evaluation of Liposomal and Crude Antigen Vaccines Against Avian Pathogenic Escherichia coli in Broiler Chickens

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Abstract

General Background: Avian pathogenic *Escherichia coli* (APEC) remains a major challenge in poultry production, necessitating reliable vaccination strategies. **Specific Background:** Liposomal and crude antigen vaccines represent distinct formulations with different capacities to stimulate humoral and cellular immune responses. **Knowledge Gap:** Comparative evidence on their immunogenic profiles in broiler chickens, particularly across key cytokine markers and IgY levels, remains limited. **Aims:** This study evaluated and compared the immunogenicity of liposome-based (LIPO) and crude antigen (CRU) vaccines against APEC in broiler chickens. **Results:** Both vaccines significantly increased IL-4, IL-10, IgY, IL-12, IL-1β, and IFN-γ compared to the control ($p < 0.0001$). LIPO vaccination induced higher IL-4, IgY, IL-12, IL-1β, and IFN-γ levels, indicating strong activation of both Th2-mediated humoral and Th1-mediated cellular responses. CRU vaccination showed increased IL-10 and moderate cytokine responses, suggesting partial immune activation. **Novelty:** This study provides a direct immunological comparison of liposomal and crude antigen formulations using multiple cytokine indicators in a controlled broiler model. **Implications:** The findings support liposomal vaccines as a promising strategy for inducing balanced immune responses against APEC, contributing to improved poultry health management and reduced reliance on antimicrobial interventions.

Highlights:
- Liposomal Formulation Induced Higher Cytokine and Antibody Responses Across Measured Markers
- Crude Antigen Vaccine Showed Partial Activation With Elevated IL-10 Levels
- Both Formulations Triggered Innate and Adaptive Immunity in Broiler Models

Keywords: Liposomal Vaccine, Crude Antigen, Broiler Chickens, Humoral Immunity, Cellular Immunity.

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Introduction

Colibacillosis is a great economic challenge of the poultry industry. The mortality, low feed efficiency, a drop in egg production, carcass condemnation, and the expense of antimicrobial treatment of the disease all cause losses, which is why the problem of antimicrobial resistance in the avian pathogenic. E. coli (APEC) isolates can be addressed only by effective management practices [1]. Vaccination is the most effective and least expensive preventive action in management of infectious diseases. To prevent the infection of APEC in chicken, a variety of types of vaccines have been developed, including inactivated vaccines, live attenuated, and subunit vaccines [2]. Liposomal vaccines hold a

potentially decent alternative. These vaccines are microscopic in nature and spherical in shape. The structures consist of an aqueous center, enclosed by lipid bi-layer. The liposomes have been classified based on size, lamellarity, surface charge, and composition. Liposomes are usually prepared using natural biodegradable lipids, and they are usually regarded as safe to use in therapeutic settings [3]. On the other hand, crude antigen vaccines are literally extracted directly out of the organism or its components, and do not undergo a lot of purification. These vaccines incorporate target antigen, as well as, other parts of the cell, whereas purified or recombinant antigens undergo purification to remove contaminants and enhance specificity. [4]

Simple crude antigens can produce strong immunological responses even though they are simple partly due to their adjuvant like constituents, which stimulate the immune system. In order to determine the protective potential of different vaccine formulations, the immunological response triggered by these different vaccine formulations is to be evaluated. Surprisingly, a significant rise in the level of interferon-gamma (IFN- g) following crude antigen vaccination is a good indication of strong Th1-mediated immune response, which is consistent with the role of IFN- g in developing cellular immunity. [5]. These findings demonstrate the relevance of comparative studies aimed at establishing the most effective immunization strategies to provide long-term immunity against APEC.

The aim of the. research that was. Conducted was to compare and assess the immunogenicity of two vaccine preparations, liposome-based and crude antigen, against (APEC).

Material and methods

Bacterial isolate: The isolate of APEC that was employed in the study was sourced Kindly [6].

Liposomal and Crude antigen Vaccine preparation:

Bacterial isolate was treated with formalin to form whole-cell antigen. After incubation, the culture broth was treated with 3.7% formalin for 18 hours. with phosphate-buffered saline (PBS) The inactivated bacteria were washed four times and used as antigen [7].

Preparation of Crude antigen Vaccine

The Suspension of APEC in phosphate buffer saline in concentration 1.5×108 CFU/ml was sonicated. During Sonication method the suspension was Kept in ice to prevent high temperature of sample, the sonication apparatus was set on 5cycle of 60 second pulse [8].

Preparation Liposomal Vaccine

The lipid biofilm was prepared by mixing methanol & chloroform (1:2 v/v) with 0.02 g cholesterol and 0.08 g phospholipid, the solvent was removed by rotary evaporators forming thin lipid film. 10 mg/ml of the crude antigen in PBS solution was add to the dried lipid layer to rehydrate the lipids. The mixture was sonicated (3 time for 20 sec) to reduce their size and achieve unilamellar vesicles. To evaluated the liposomes size the suspension was examined by Dynamic Light Scattering and Transmission Electron Microscope.

Preparation of Crude antigen and Alum Hydroxide Gel (Al hydrogel)

Alum hydroxide gel, forty grams of sodium hydroxide (NaOH) dissolved in 1.0 L of DW to prepare the alkaline solution, which was subsequently mixed with 1.0 L of a 10% (w/v) potassium aluminum sulfate (alum; $K_2SO_4 \cdot Al_2(SO_4)_3 \cdot 24H_2O$) solution commonly known as aluminum hydroxide adjuvant was prepared by controlled precipitation of aluminum salts. Typically, aluminum potassium sulfate (alum) was dissolved in water and then treated with an alkali such as sodium hydroxide under controlled pH (around 6.0–7.0) [9]. The precipitate of aluminum hydroxide was allowed to mature and then washed to remove residual salts. The resulting gel is a colloidal suspension of hydrated aluminum hydroxide [10].

Animal Model

Innate and adaptive immune responses were evaluated using broiler chickens as an experimental paradigm. The animals were randomly divided into three experimental groups G1 (negative control), G2 (liposomal vaccination), and G3 (crude antigen vaccine). The chickens had a one-week pre-experimental period in the plastic cages after having access to food and water. Animals were maintained under stable conditions and temperatures and husbandry methods were maintained all through the research. The ethical principles of Basra University in the collection and treatment of samples and the care of animals as defined by the Institutional Animal Care and Use Committee (IACUC) were strictly observed in every

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experimental procedure and in accordance with the accepted ethical standards [11].

G2 chickens were subcutaneously injected with 0.5 mL liposomal vaccine (1.5 x 108 CFU/mL crude antigen vaccine) and G3 chicken injected with the same dose of crude antigen vaccine. A subcutaneous injection of 0.5 mL of sterile normal saline was given to the negative control group (G1). Vaccinations were administered at 10, 20, and 30 days of age. For immunological testing, blood samples were taken from each group on day 35 of hatching.

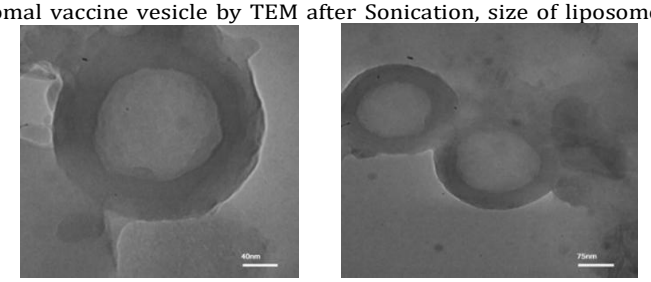
Measurement of immune indicator

The following Chicken Cytokines IL-4, IFN-γ, IL-10, IL-1β, IL-12 were measured by using ELISA. Kit from (Sun. long. Biotech Co. LTD).The Igy concentration Measured by using Elisa. Kit from (Sun. long. Biotech Co. LTD).

Result

After Cultivation of E coli on EMB medium the bacteria showed the metallic shin in Eosin- methylene blue Agar . The two vaccines (LIPO &Crude) were made in the laboratory and the chickens were injected with three subcutaneous doses of 0.5ml doses (1.5×108CFU/ml). In periods (10,20 and 30 days)Blood samples were collected on day 35th. Cytokines were measured using ELISA.

Figure (1) Detection of liposomal vaccine vesicle by TEM after Sonication, size of liposomes range from 40 nm to 75 nm



Serological Tests (ELISA test)

The cytokine concentration of vaccinated chickens in Table (1) & Figure (2,4-B).

Table (1): Humoral immune (IL-4, IL10, IgY) for two types of vaccine compared with N .C

Table (1) presents the mean (M) and standard deviation (SD) values for humoral immune markers IL-4, IL-10, and IgY across negative control (N.C.), liposomal (LIPO), and crude vaccine groups, revealing distinct immunomodulatory profiles in poultry models.

Type of cytokine	N.C	LIPO	Crude	Significance
IL-4 M ± SD	11.56 ± 0.4127	18.10 ± 0.5319	14.87 ± 0.5243	P < 0.0001
IL-10 M± SD	24.99 ±0.4952	45.71±0.9739	76.39±0.7581	P < 0.0001
IgY M ± SD	3.664±0.4370	40.73± 0.4633	35.16±0.4278	P < 0.0001

The cytokine concentration of vaccinated chickens in Table (2) & Figure (3,4-A).

Table (2): Cellular immune (IL12, IL-1B, IL-1B) for two types of vaccine compared with N .C.

Table (2) displays mean (M) and standard deviation (SD) values for cellular immune markers IL-12, IL-1β, and IFN-γ across negative control (N.C.), liposomal (LIPO), and crude vaccine groups, highlighting divergent Th1 activation profiles in poultry vaccination.

Type of cytokine	N.C	LIPO	Crude	Significance
IL12 M ± SD	8.522±0.4260	27.80±9.657	8.257±0.3416	P < 0.0001
IL-1B M ± SD	20.15±0.4352	62.06±0.4412	31.05±1.055	P < 0.0001
IFN-γ M ± SD	10.35±0.4157	29.56±0.5515	12.59±0.4986	P < 0.0001

Serological Tests by ELISA technique were explained in Figure (2) ,(3),(4).

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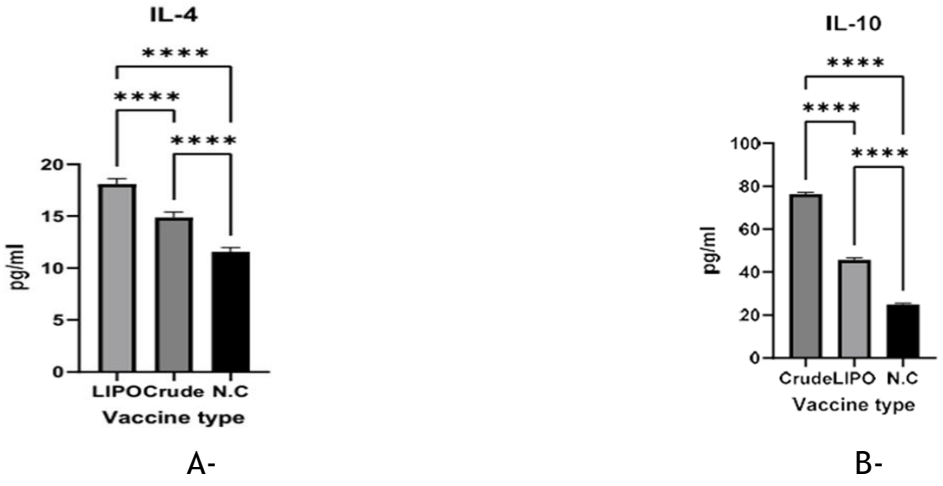
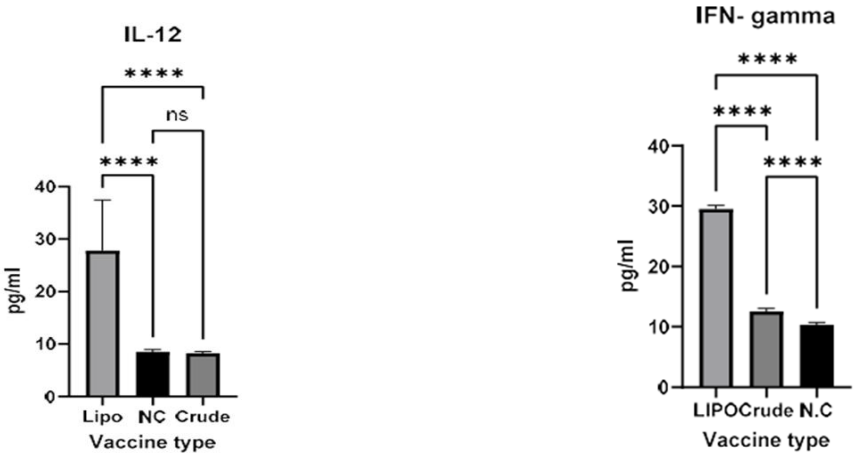
Figure (2)-A-Concentration of IL-4 in all groups (LIPO&Crude)compared with the -N.C group, B- Concentration of IL-10 in all groups (LIPO.Crude)-compared with -the N.C group



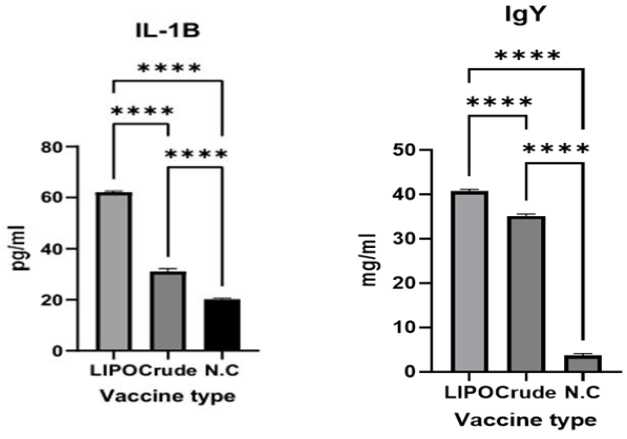
A- B-

Figure (3)-A-Concentration of IL-12 in all groups (LIPO&Crude)-compared with the N.C -group, B- Concentration of IFN-gamma in all groups (LIPO.Crude)-compared with the N.C group



A - B-

Figure (4)-A-Concentration of IL-1B in all groups (LIPO&Crude)compared with the- N.C group, B- Concentration of IgY in all-groups(LIPO.Crude) compared- with the N.C group



A- B-

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Discussion

Data represent immune response measurements for three groups: NC (negative control), LIPO (liposome-based vaccine), and CRU (crude or surrogate vaccine). Six immune parameters associated with immune response were measured with their means (M) and standard deviations (SD) presented along with significance testing (P-values). statistical analysis by one-way ANOVA.IL-4 and IL-10, IgY related to anti-inflammatory and humoral responses,. Reflects humoral antibody response [12]. These results affirm liposomal vaccines' edge in eliciting robust, quantifiable humoral responses, as elevated IL-4 and IL-10 correlate with superior IgY levels essential for pathogen neutralization in poultry these result similar to that records by [13]. These results affirm the edge of Liposomal vaccines in eliciting robust, quantifiable humoral responses as evidenced here, consistently augment IL-4 and IL-10 alongside IgY, aligning with recent findings on their capacity to sustain antigen presentation and foster balanced humoral immunity in avian species [14]. Increase in IL-4 after the vaccination facilitates the differentiation of Th2 cells, B-cell stimulation and secretion of antibodies (e.g IgY) which increase humoral immunity and vaccine efficacy, however excessively suppresses effector T cells and immunity. The ratio of IL-4/IL-10 changes is optimal and the IL-4 produces the most protective Effect While high IL-10 may suppress this [15]. Liposomal preparations show superiority in the amplification of IL-12 and IFN- gamma, which are major inducers of the NK and CD8+ T-cells during the clearance of intracellular pathogens in avian models, and low SDs indicate coherent polarization of the Th1 response. This is in line with recent avian research with liposomal adjuvants increasing IL-12/ IFN-gamma synergy, which increased splenocyte proliferation and post-vaccination protection against fowl cholera. Crude extracts, however, yielded negligible IL-12 despite IL-1β elevation, implying decoupled inflammasome activation without sustained Th1 commitment, a pattern

linked to tolerance in unrefined antigens [16]. Figure 2(a) shows the concentration of IL-4 all groups (LIPO and crude) compared to the N.C group. Using Bonferroni's Multiple Comparison Test, IL-4 shows an increase in Lipo compared to the negative group, and there is also a significant increase in crude compared to the negative group. When comparing Lipo and crude, Lipo is the best in raising the immune response in poultry. Figure 2(b) shows the concentration of IL-10 in all groups (LIPO and crude) compared to the N.C. group. Using Tukey's Multiple Comparison Test IL-10 acts as an anti-inflammatory cytokine that regulates innate immune responses to prevent excessive inflammation. Produced by innate lymphoid cells and other innate cells, it suppresses pro-inflammatory cytokine production like IL-1β.IL-10 levels are higher in the Lipo group compared to the negative group, and there is also a significant increase in the crude group compared to the negative group. When comparing Lipo and crude, the crude group is superior in eliciting an immune response in poultry due to the presence of Alum. Figure 3(a,b) shows the concentration of IL-12 and IFN-gamma in all groups (LIPO and crude) compared to the N.C. group, and the higher concentration of the liposomal vaccine compared to the crude formulations and negative controls. IL-12 and IFN-gamma levels were higher in the Lipo vaccine compared to the negative control group. Crude levels were also significantly higher compared to the negative control group. When comparing Lipo and crude vaccines, Lipo demonstrated the superior effect in eliciting the immune response in poultry. Figure 4(a) shows the concentration of IL-1B in all groups (LIPO and crude) compared to the N.C. group IL-1β serves as a key pro-inflammatory cytokine in innate immunity It promotes inflammationand the higher levels of the liposomal vaccine compared to crude formulations and negative controls. Using Bonferroni's multiple comparison test, the results showed that when comparing N.C. versus LIPO, LIPO was superior; when comparing N.C. versus crude vaccines, crude vaccine appeared superior more over when comparing both vaccines together, LIPO was the superior

vaccine The figure Figure 4(B) shows that both vaccinated groups (LIPO and Crude) induced markedly higher serum IgY concentrations than the negative control (N.C), with all pairwise comparisons reaching very high statistical significance (****, $p < 0.0001$). The LIPO group exhibits the highest IgY concentration, around 40 mg/ml, indicating strong humoral stimulation by the liposome-based formulation. The Crude vaccine group shows slightly lower but still robust IgY levels (≈35 mg/ml), whereas the N.C group remains near baseline (≈3–4 mg/ml), confirming that specific vaccination these result similar to that reported by [17].

Data from various chicken breeds suggest that total IgY concentration in adult chicken serum averages around 3.2 to 26.9 mg/ml with a mean near 7.1 mg/ml, but in young chicks notably less due to development stage. This general range aligns with observations in commercial broiler breeds like Rose 308, which are expected to have similar immunological profiles during early development [18]. The results also showed that all, vaccines elicited both innate and adaptive immune responses [19] [20].

Conclusions

There was measurable anti-APEC immunity following either crude antigen or liposome-based vaccination in the present study, but the latter formulation is a more immunogically potent and effective vaccine against APEC in broiler chickens. The results showed that compared to the controls, liposomal vaccination elevated the levels of humoral immune markers such as IL-4, IL-10, and IgY substantially, while also inducing a strong cellular immune status as observed by enhanced IL-12, IL-1β and IFN-γ levels consistent with Th1 and Th2 immune polarization. In contrast, the crude antigen vaccine elicited comparatively weaker and less coordinated immune responses which may indicate partial immune activation and potential immuomdulatory limitations resulting from unrefined antigen(s). These data suggest the improved ability of liposomal delivery systems to amplify antigen delivery, delay cytokine signaling, and broaden the potency and duration of both innate and adaptive immunity. The results of this work is of great importance for poultry health management and liposome-based vaccines can be advantageous to increase the protection against colibacillosis without or less contribution for utilizing of antimicrobial agents, thus minimizing the development of antimicrobial resistance. Moreover, technological advances in vaccination strategies, including the implementation of modern vaccine delivery platforms, appear to be beneficial to flock performance and may help with long-term economic sustainability of the poultry sector. Longer studies in field environments will be needed to further assess the duration of protective efficacy of the liposomal vaccines, as well as to investigate appropriate dosing, delivery mechanisms, and cross-protection against a variety of APEC strains, in addition to the feasibility of commercial scale up to support practical application in commercial poultry production systems.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Data availability

All data are included in this manuscript

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