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Research Article

Valuable systemic immunity for lambs immunized intranasally with sheep pox virus encapsulated in chitosan nanoparticles

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Abstract
Sheep pox virus (SPXV) from the Poxviridae family causes a highly contagious and drastic disease in sheep. Periodic outbreaks are mainly confined to Asia and Africa, however also new emergence has recently been reported in other parts of world. The necessity for a new vaccine is required to achieve effective and high coverage vaccination for sheep flocks. For efficient mucosal vaccine delivery, chitosan nanoparticles with mucoadhesive properties have the ability to open tight junctions between epithelial cells, and facilitate antigen uptake. Current study was conducted to evaluate protective efficacy of live attenuated sheep pox virus encapsulated in chitosan nanoparticles (LSPV-CS-NPs). Thirty immunological naïve lambs aliquot divided into lambs given Phosphate buffer saline as control group, second group with blank chitosan nanoparticles, third group intranasal vaccinated with LSPV-CS-NPs, fourth group subcutaneously vaccinated with LSPV-CS-NPs and fifth group contains lambs immunized with commercial vaccine subcutaneously. Post immunization, by using ELISA technique, the sera was investigated for specific sheep pox virus IgG and IgA, the assay showed significantly increasing in third group compared to other groups over the course of eight weeks. Altogether, the CS-NPs could be considered as an efficient intranasal LSPV delivery system for nasal vaccines to enhance systemic immunity, therefor this approach has an immense application promise.

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Introduction

Sheep pox virus (SPXV) is a type of *Capripoxviruses*, causing serious infection in sheep that threatens the livestock industry worldwide, SPXV appears periodically in sheep flocks, especially in lambs, and which varies in severity from chronic to acute, usually the affected sheep characterized by appearance lesions on skins and mucous membranes (1). Periodic outbreaks are mainly confined to Asia and Africa, but also new emergence has recently been reported in other parts of world (2). Sheeppox disease and another infection of the genus in the same family of Poxviridae called ORF virus, both of which are highly prevalent and periodically cause severe loss of ovine flocks in the Iraqi field (3, 4, 5, 6). Currently, SPXV vaccines are administered by parenteral routes. However, some drawbacks related with those administration for SPXV vaccine leads to appear sheep pox disease from time to another (7).

Nowadays with cumulative findings, the mucosal vaccination has been considered successful alternative to induction robust mucosal immune response, in addition to systemic response (8). Mucosal tissues have been reported to provide unique sites for indication robust humoral and cellular immune responses. Mucosal associated lymphoid tissues (MALT) serve as major sites for circulating and communicating integrated mucosal immune cells. Nasopharynx-associated lymphoid tissue (NALT) is an ideal site can be considerable for intranasal vaccination (9). The nasal and oral vaccine administrations are most effective routes of mucosal vaccination that have extensively been investigated in comparison with other routes. Nasal vaccines have the potential to induce immune responses at the site of entry for most lung pathogens. Given the relatively large surface area is available for interaction with the vaccines used (10). On the other hand, the recent advice by Wolff et al. in 2022 has confirmed the nasal vaccination route is preferable (11). Furthermore the immunity elicited via this rout is qualitatively and quantitatively different from those elicited by other routs (12). However, most antigens have low affinity for the nasal epithelium with a rapid clearance rate, due to a viscous nature of mucus with cilia activity as a motivator, may not allow sufficient retention for antigen to be taken up by APCs in NALT (13). Nanotechnology has been served to solve thus issue by modifications of vaccine's formula with suitable nanoparticles (NPs) contribution. In this regard, a vaccine protected with nanomaterial that helps to reach inaccessible sites with sustainable release, such nasal cavity has long been tested and discussed in several publications. In general, selection of appropriate nanoparticles is backbone of the success of any proposed candidate in mucosal vaccine. Chitosan is one of the popular polymers, which has been investigated in many trails and confirmed possess desired properties. Chitosan and its derivatives have been extensively explored to evaluate their efficacy in eliciting immune response. Chitosan nanoparticles (CS-NPs) represent an efficient vaccination tool, due to the unique ability to mucoadhesion caused by the electrostatic interaction between cationic molecular chain and negative charge of cell surface and mucus (14). In current study, vaccine of sheep pox virus attenuated and live encapsulated in chitosan nanoparticles LSPV-CS-NPs were previously prepared in our lab and was assessed for tissue culture Infectivity. We evaluated the specific humoral immune responses of lambs vaccinated with LSPV-CS-NPs. Post vaccination, an ELISA assay was performed to measure specific IgG and IgA in the serum of experimental lambs.

Materials and methods

Animals of study

A healthy 30 Awassi sheep in four months old were kept naïve with extremism of sterilized environment. These lambs were divided into groups and subjected under immunization protocol. Enrolled lambs according groups were inoculated with the different formulations by intranasal or parental administrations as shown in Table 1. All studies of experiment were conducted and evaluated in accordance with the Animal Ethics Committee of Basra University, Iraq.

Dosage of Vaccine

The LSP-CS-NPs formulation was produce through ionic gelation method of live attenuated SPXV loaded with chitosan nanoparticles in previous study by AL-Zubaidi, Thwiny and AL-Biati (in press) (15), this vaccine candidate was titered and evaluated $10^{7.32}$ per mL as tissue culture infectious dose (TCID) through Reed-Meunch method by same authors in another study (at processing) (16). Whereas the commercial vaccine from Al Kindi for veterinary Drugs and Vaccines Company had a titer TCID 10^{6} per mL according instructions of manufacture.

Table 1: Methodology of animals study and Immunization protocol

Vaccination for all lambs in groups with dose volume 0.5 ml						
Lamb Groups	PBS	Blank CS-NPs	LSPV-CS-NPs	Commercial vaccine	i.n.	s.c.
G1	+	_	_	_	_	+
G2	_	+	_	_	+	_
G3	_	_	+	_	+	_
G4	_	_	+	_	_	+
G5	_	_	_	+	_	+

G1: lambs given PBS as control, G2: vaccinated intranasal with blank CS-NPs, G3: vaccinated intranasal with LSPV-CS-NPs, G4: subcutaneously vaccinated with LSPV-CS-NPs and G5: lambs immunized with commercial vaccine subcutaneously. i.n.: intranasal administration, s. c. subcutaneously administration. + Symbol was denoted the type and rout treatments of group, while - symbol denoted no treatment.

Serum preparation

Five milliliter of blood samples from jugular vein were collected at the end of 2nd 4th, 6th and 8th weeks post-vaccination, and allowed to clot by leaving undisturbed at room temperature 15 minutes, then centrifuged at 3,000 rpm 20 minutes to remove clot, then cleared serum samples were dispensed into Eppendorf-tubes by using micropipette, and properly labeled with ID and date to use in next serological investigation with ELISA.

Enzyme-linked immunosorbent (ELISA) assay

Herein, the ELISA a technique used to determine concentrations of specific sheep pox antibodies included IgG and, IgA, after different vaccine formulations was inoculated for enrolled lambs according groups. ELISA kits subjected herein were obtained from SunLong Biotech Co., LTD. And the procedure for assay was done according the manufacture instructions (17).

Results

Quantitation specific SPV-IgG response

Figure (1) exhibited the continuity of specific SPV-IgG levels in immunized lambs for experiment groups. Post immunization, all lambs enrolled in groups 3, 4 and 5 showed SPV-IgG production in response to vaccine formulations over the course of 2 weeks up the end of trail week. Fourth week post immunization was showed highest response in serum lambs immunized subcutaneously with commercial vaccine. However, lambs immunized intranasally with LSPV-CS-NPs showed significant increase of the specific SPV-IgG levels eighth week p.i compared to other groups. Remarkably, there was no response for lambs given PBS subcutaneously or blank CS-NPs intranasally for the length of the experiment, except screening very low concentrations of SPV-IgG were detected for two lambs of each G1 and G2 in second week p.i. Indeed, this sample of both groups was used as a baseline and not expected to show response. In comparing effect of time progression inside each group, the results showed that weeks' time 8 presented a significant increase of SPV-IgG level ($p<0.0001$), when it is compared with weeks' time 2, 4 and 6 inside G3. Lambs subcutaneously vaccinated with LSPV-CS-NPs showed an increase in SPV-IgG concentration after second week throughout the fourth and sixth week but indicated a decline in the eighth week.

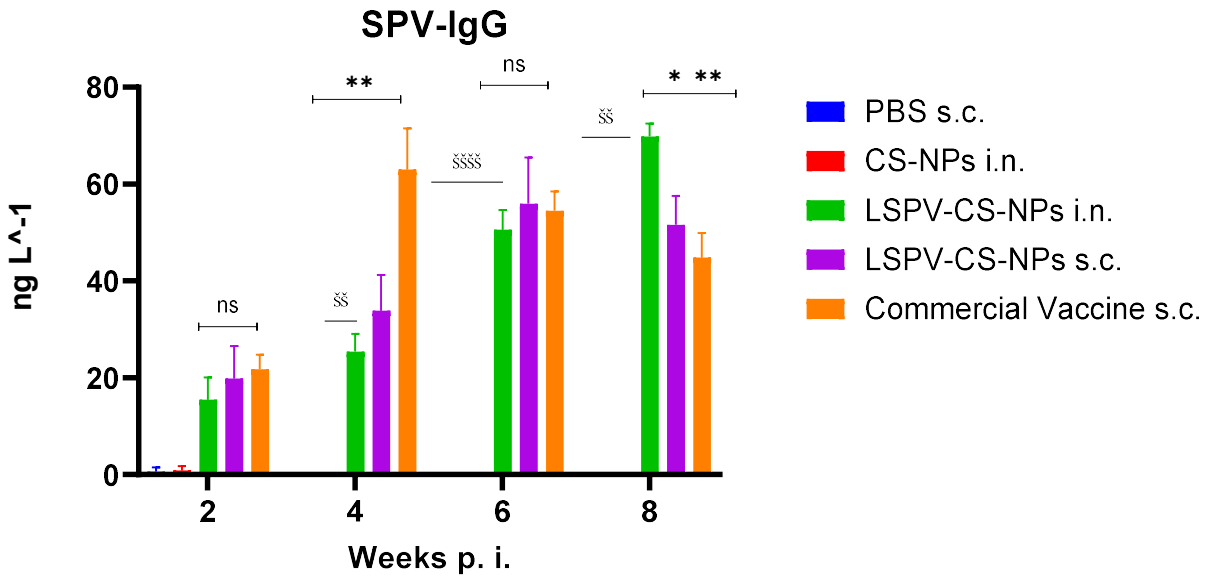

Figure 1: Serum concentration of SPV-IgG in nanogram per liter of immunized lambs. Each value for groups in the graph represents means standard deviation of 6 replicates with Significance value under P ≤ 0.05. wp.i: weeks post-immunization; Asterisks (*) indicate significant differences among vaccine groups at each time point; section marks (ŝ) indicate significant change from the effect of time progression inside each group; ns: no significance; s.c: subcutaneously; i.n: intranasally.

4.2.1.2. Quantitation specific SPV-IgA response

Lambs were nasally administered LSPV-CS-NPs elicited higher IgA concentration than other experimental lambs (figure 2), whereas the other vaccines did not show significant effects on the IgA response. None of the formulation induced substantial IgA levels. After subcutaneous immunisation, LSPV-CS-NPs induced the production of significantly more IgA compared to a solution of commmercial vaccine, but no additional effect of the encapsulation of adjuvants was observed. However, CS nanoparticles containing LSPV significantly boosted the IgA production ($p<0.001$). In early response, the second and fourth week of experiment represented a non-significant effect among the lambs in the last three groups.

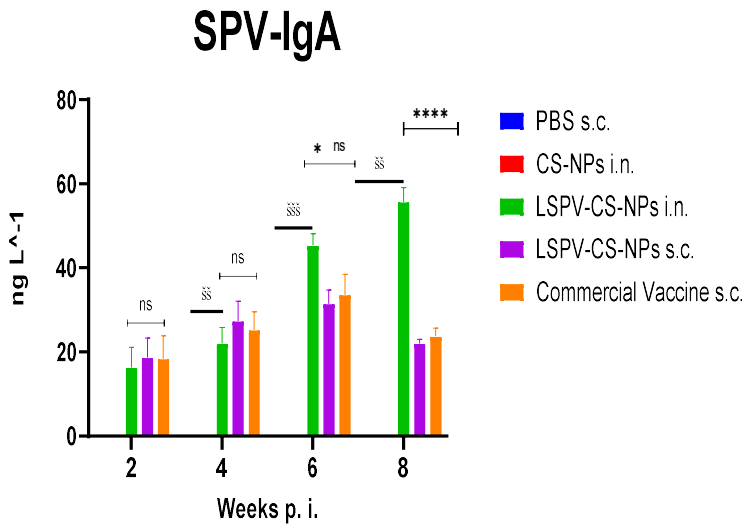

Figure 2: Serum concentration of SPV-IgA in nanogram per liter of immunized lambs. Each value for groups in the graph represents means standard deviation of 6 replicates with Significance value under $P \leq 0.05$. wp.i: weeks post-immunization; Asterisks (*) indicate significant differences among vaccine groups at each time point; section marks (ŝ) indicate significant change from the effect of time progression inside each group; ns: no significance; s.c: subcutaneously; i.n: intranasally.

Statistical analysis

GraphPad Prism version 8 was used to perform the statistical analysis. Also, two-way analysis of variance (ANOVA) and Tukey's multiple comparison test were used to analyze the obtained data. Data were showed as mean ±standard deviation (SD).

Discussion

Inducement of mucosal immunity involves a distinctive interaction series of antigens with immune cells that ultimately leads to unique immune responses. These responses basically correlated with the action of specialized epithelial cells called micro fold cells (M cells), which organized intermittently in the follicles associated epithilium (FAE) of (MALT) (18). This location of M cells is qualified capture antigens then deliver them to the underlying professional (APCs) such as dendritic cells, microphage, B cells or even epithelial cells, where they efficiently processed the antigen and displayed it as small fragment on MHC molecule, which eventually results into activation of specific T and B lymphocytes, in turn, they are imported to the effector sites. Primed lymphocytes develop further maturation and expansion of immune responses, which can take place in lymph nodes. Antigen committed lymphocytes differentiate to turn into effector/ memory T and B cells prior to its homing to mucosal sites. These specialized cells are standing by to pick up the identified antigen that traffic back in mucosal tissue, and often respond vigorously to invaders to provide protection at the main sites of infectious threat: the mucosae. The induction of a specific mucosal response is regulated as immune cells and antibodies (19).

In current study, all vaccine formulations had a pronounced and lasting immune response with formation of IgG and IgA antibodies in serum irrespective of the vaccination protocol. The results shows a variable IgG and IgA concentrations in immunized lambs, and that attributed to vary in the formulations and administration of vaccine. Antibody levels in G4 and G5 were recorded maximizing peak at first 2 wpi for commercial vaccine, later they appeared to drop moderately. However, this reached statistical significance only for IgG antibodies in the third group. The nasal LSPV-CS-NPs group showed also a trend ($p = 0.05$) towards lower levels at early response compared to subcutaneously groups after that IgG concentrations increasing to recorded highest level in delayed response stage at eighth wpi.

Regarding the other important systemic antibody IgA, the production IgA was less than IgG concentrations for all immunized lambs over a course the experiment and these results constant with most previous studies (20, 21). The IgA concentrations for G4 and G5 increased in middle response stage at 4 and 5 weeks pi, then commence to decline at delayed response stage. In similar to concentrations of IgG, The IgA concentrations in sera of G3 increased linearly as the experiment progressed and outperformed all other sera from experimental lambs upon delayed response stage.

These findings differ in somewhat with several a previous trails in which humoral immunity was assessed of total immunoglobulins by SNT and ELISA (22, 23). A log neutralization index of ≥1.5 is considered positive for Capripoxviruses, also pre-existing neutralizing titres <1:32 are being more susceptible to pox infection than those with antibody titers ≥1:32 (24). However, the level of antibodies that confers protection against poxviruses is unknown. The discrepancies between our results and these studies in the timing and measurement of humoral response can be explained by the use different assessment procedures and virus strains or species and breed of animals, moreover the conditions of each experiment play significant role and effect in assessment the vital parameters of animals. Therefore, in dependent on the accurate assessments in current study for specific antibodies response, another possible consideration may emerge that the actual quantification of whole Igs containing other unknown or known proteins respond to classical assays in their Ig-like behavior, however more studies with developed serological tools are required.

From another angle, our results were consistent with modern study was conducted to evaluate the humoral response to inactivated SPXV strain as antigen in indirect ELISA for serum of infected sheep, as after 30 dpi reported highest reconversion of antibodies (25). Another most modern study by Cheng with others in 2023 confirmed our finding when cows were immunized intranasally against bovine viral diarrhea virus, and found a delayed and robust antibody responses extending up to seven weeks and continuing to increase, when they were looking for a specific IgG and IgA response (26).IgG and IgA antibodies of G3 appear in a potent existence rather late during experimental period, which is in contrast with lambs given vaccine formulations subcutaneously enrolled in G4 and G5, where IgG and IgA antibodies were detected in higher levels as early as 4 and 6 weeks p.i. respectively. One explanation is that the kinetics of antibodies in sera of G3 were higher than that in other immunized groups, indicating that these antibodies are the result of leakage of local antibodies into the circulation (27). Another explanation may be the leakage of mucosal vaccine through mucosal tissue into the circulation and this would aid in development systemic responses of lambs vaccinated intranasally (28).

In conclusion we can infer that the vaccine formulation LSPV-CS-NPs administrated via nostrils of lambs takes longer to thrive in mucosae by aiding mucosal adhesion property of chitosan cross mucosal barriers, as adaptive response events to induction in mucosal tissues require more of time than systemic adaptive immune responses. Whatever, if stimulation occurred, these mucosal responses lead to persistence of effector immune cells and molecules. This study supported the nasal vaccination and confirmed the potential of the vaccine candidate LSPV-CS-NPs to induce

systemic immune response, thus further studies in mucosal vaccines are required before applying in the fields.

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Conflict of Interest

The author(s) declared that there is no conflict of interest.

References

1. Tuppurainen E.S.M., Venter E.H., Shisler J.L., Gari G., Mekonnen G.A., Juleff N., Lyons N.A., De Clercq K., Upton C., Bowden T.R., S. Babiuk , L. A. Babiuk. Review: Capripoxvirus Diseases: Current Status and Opportunities for Control. *Trans. Emerg. Dis.* 2017;64:729–745.
2. Biswas S, Noyce RS, Babiuk LA, Lung O, Bulach DM, Bowden TR, Boyle DB, Babiuk S, Evans DH. Extended sequencing of vaccine and wild-type capripoxvirus isolates provides insights into genes modulating virulence and host range. Transbound Emerg Dis. 2020 Jan;67(1):80-97.
3. Mayea KQ, Thwiny HT, Ayyez HN. Genetic Phylocomparative Analysis of B2L, F1L Genes in Orf Virus Isolated from Felid Infected Sheep. Bas J Vet Res. 2023;22(1):47-55.
4. Mohammed YJ, Yousif EH. Molecular detection and dermato pathological analysis of ORF virus infection in sheep and goats in basrah province. IJAS. 2022 Jun 29;53(3):611-24.
5. Al-Mubarak F, Thwiny H, Hizam M. Seroprevalence of Orf virus in sheep in Basrah province, Southern Iraq. Bas J Vet. 2022 Dec 1;21(S1):58-66.
6. Noah IA, Rasheed SA. Effect of pox vaccine on blood picture in adult ewes. Bas J Vet. 2020;19(3).
7. Zangana IK, Abdullah M.A. Epidemiological, clinical and histopathological studies of lamb and kid pox in Duhok, Iraq. Bulgarian J Vet Med. 2013;16: 133-138.
8. Hellfritzsch M. and Scherließ R. Mucosal Vaccination via the Respiratory Tract. Pharmaceutics. 2019;11(8):375.
9. Brandtzaeg P. Potential of nasopharynx-associated lymphoid tissue for vaccine responses in the airways. Am J Respir Crit Care Med. 2011 Jun 15;183(12):1595-604.
10. Teeling KP, Werling D & Berner D. Preliminary Volumetric Calculation of the Mucosal Surface in the Nares of Lambs Using a Segmentation of Computed Tomographic Images. Front Vet Sci. 2020;18 (7):620-647.
11. Wolff J, Abd El Rahman S, King J, El-Beskawy M, Pohlmann A, Beer M, Hoffmann B. Establishment of a Challenge Model for Sheeppox Virus Infection. Microorganisms. 2020 Dec 15;8(12):2001.
12. Celma CC, Boyce M, van Rijn PA, Eschbaumer M, Wernike K, Hoffmann B. and et al. Rapid generation of replication-deficient monovalent and multivalent vaccines for bluetongue virus: protection against virulent virus challenge in cattle and sheep. J Virol. 2013;87:9856–64.
13. Wallis J, Shenton DP. and Carlisle RC. Novel approaches for the design, delivery and administration of vaccine technologies. Clin Exp Immunol. 2019;196:189–204.
14. Illum, L., Jabbal-Gill I, Hinchcliffe M, Fisher AN, Davis SS. Chitosan as a novel nasal delivery system for vaccines. Adv Drug Delivery Rev. 2001;51(1):81–96.
15. AL-Zubaidi, M.R.K., Thwiny, H.T. and AL-Biati, M.N. Modulation of chitosan nanoparticles properties for sheep pox mucosal vaccine delivery with cytotoxicity and release studies. Iraqi J Vet Sci. 37(1): 2023/12/01. Article ID#: 000-23 [IJVS-2682].
16. AL-Zubaidi, M.R.K., Thwiny, H.T. and AL-Biati, M.N. Evidence of Specific-secretory Immunoglobulin A in Response

to Nasal Immunization with Chitosan Nanoparticles based Sheep pox Vaccine in Lambs. Basrah J Vet Res. In processing.
17. Hasan Lafta M, Kadhum Muhsen R. Seroepidemiological study of ovine Q fever in Basra Province, Iraq. Basrah J Vet Res. 2016 Jun 28;15(1):241-8.
18. Kozlowski PA, Mantis NJ, Frey A. Mucosal Vaccination: Strategies to Induce and Evaluate Mucosal Immunity. Frontiers in Immunology. 2022:2017.
19. Brandtzaeg, Per. Mucosal Immunity: Induction, Dissemination, and Effector Functions. Scandinavian J Immuno. 2009: 70.
20. Zhao K, Chen G, Shi XM, Gao TT, Li W, Zhao Y, Zhang FQ, Wu J, Cui X, Wang YF. Preparation and efficacy of a live newcastle disease virus vaccine encapsulated in chitosan nanoparticles. PLoS One. 2012;7(12):e53314.
21. Zhao K, Sun B, Shi C, Sun Y, Jin Z, Hu G. Intranasal immunization with O-2'-Hydroxypropyl trimethyl ammonium chloride chitosan nanoparticles loaded with Newcastle disease virus DNA vaccine enhances mucosal immune response in chickens. J Nanobiotechnology. 2021 Aug 11;19(1):240.
22. Tian, H., Chen, Y., Wu, J. *et al.* Serodiagnosis of sheeppox and goatpox using an indirect ELISA based on synthetic peptide targeting for the major antigen P32. J Virol. 2010;7: 245.
23. Rhazi H, Mikou K, Sadeqy Y, Alhayane M, El Mejdoub S, Safini N, Lenk M, Tadlaoui KO, Elharrak M. Evaluation of ELISA and VNT for sheeppox virus antibody detection and development of an immunoenzymatic quantitative method. J Immunol Methods. 2022 Mar;502:113226.
24. OIE. (2019). Lumpy skin disease, Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2019. Author.
25. Berguido F.J., Gelaye E., Liu, Y., Davaasuren B., Krstevski K., Djadjovski I., Ivanova E., Goujgoulova G., Loitsch A., Tuppurainen E. and et al. Development and Optimization of Indirect ELISAs for the Detection of Anti-Capripox virus Antibodies in Cattle, Sheep, and Goat Sera. Microorganisms. 2022;10:1956.
26. Cheng Y, Tu S, Chen T, Zou J, Wang S, Jiang M, Tian S, Guo Q, Suolang S, Zhou H. Evaluation of the Mucosal Immunity Effect of Bovine Viral Diarrhea Virus Subunit Vaccine E2Fc and E2Ft. *International Journal of Molecular Sciences*. 2023; 24(4):4172.
27. Lavelle EC and Ward RW. Mucosal vaccines - fortifying the frontiers. Nat Rev Immunol. 2022; 22(4):236-250.
28. Rhee JH. Current and New Approaches for Mucosal Vaccine Delivery. Mucosal Vaccines. 2020:325–56.