IN VIVO EVALUATION OF MICROENCAPSULATED BICISTRONIC PLASMID DNA VACCINE AGAINST *Vibrio cholerae*

By

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Chair : Prof. Rozita Rosli, PhD
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The aggression of cholera infection in some parts of the world is seen as one of the major public health concerns in global perspective due to its epidemic and pandemic potentials. As an approach to control the outbreak in risky regions and endemic areas, oral cholera vaccines (OCVs) which utilise conventional vaccinology strategy using killed whole-cell *Vibrio cholerae* have been recommended as a preventive measure. However, they possess several drawbacks in terms of vaccine handling and storage, logistics and cost values besides their safety concerns in high-risk individuals. As an alternative over the conventional OCVs, the concept of bicistronic DNA vaccine encoding immunostimulatory antigens which are cholera toxin subunit B (*ctxB*) and toxin coregulated pilus subunit A (*tcpA*) genes from *V. cholerae* was explored in this study. A previous study has demonstrated the potential role of bicistronic vaccine construct *in vitro* through its successful expression in COS-7 cells. However, evaluation on immunogenicity of the vaccine has not been conducted *in vivo*. Therefore, the main objective of this study was to evaluate the *in vivo* potential of the microencapsulated bicistronic plasmid DNA (pDNA) vaccine against cholera infection using removable intestinal tie adult rabbit diarrhea (RITARD) model. For a successful delivery through oral route, encapsulation of the vaccine using alginate microspheres was developed through water-in-oil emulsification with encapsulation efficiency of 88.6% and further validated through physical and behavioural characterisations of the microspheres. Successful pDNA delivery was also observed using a simulated gastrointestinal pH condition. Subsequently, oral vaccination with these alginate-encapsulated-vaccines was performed *in vivo* using seven-week-old New Zealand White (NZW) rabbits. Fifteen female rabbits were used in this study and divided into five groups: (1) Mock-infected control (negative control); (2) Empty alginate microsphere (non-vaccinated); Alginate microspheres encapsulating (3) pVAX-*ctxB*; (4) pVAX-*tcpA*; and (5) pVAX-*ctxB*-*tcpA*. Except for group 1, other test groups were challenged with *V. cholerae* of serotype Ogawa and biogroup El Tor, two weeks after the vaccination, by utilising the RITARD model. The animals were

monitored for cholera symptoms five days' post-surgery and rectal swab cultures for *V. cholerae* of Group 5 (bicistronic vaccine construct) showed negative results. On the other hand, cholera infection was not evident in the bicistronic vaccine group based on gross examination in post-mortem and histological analyses. Besides that, the cytokine expression level (TNF-α, IFN-γ, IL-10 and IL-6) was evaluated using quantitative polymerase chain reaction (qPCR) following the immunisation and infection challenge. The bicistronic group showed an increase of systemic IFN-γ and IL-10 at 12-day post-vaccination, though not significant, indicating the possible activation of both T-helper 1 and 2 types of response. However, the level of all cytokines did not change after the infection challenge. In brief, the alginate encapsulating bicistronic pVAX-*ctxB*-*tcpA* portrays a potential in inducing host immune response against cholera infection, although several improvements and comprehensive analyses are required to evaluate its role as a DNA vaccine.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENILAIAN *IN VIVO* MIKROPENGKAPSULAN VAKSIN DNA PLASMID DWISISTRONIK TERHADAP *Vibrio cholerae*

Oleh

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April 2021

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Serangan jangkitan taun di beberapa kawasan di dunia dilihat sebagai salah satu masalah kesihatan awam utama dalam perspektif global kerana wabak dan potensi pandemiknya. Sebagai pendekatan untuk mengendalikan wabak ini di kawasan yang berisiko dan endemik, oral vaksin DNA taun (OCV) yang mengaplikasikan strategi vaksin konvensional dengan menggunakan sel utuh *Vibrio cholerae* yang telah dibunuh adalah direkomendasikan sebagai langkah pencegahan. Namun, vaksin ini mempunyai beberapa kekurangan dari segi pengendalian dan penyimpanan vaksin, logistik dan nilai kos selain isu keselamatan bagi individu berisiko tinggi. Sebagai alternatif pada *OCV* konvensional, konsep vaksin DNA dwisistronik yang mengekod antigen perangsang imun iaitu gen subunit B toksin taun (*ctxB*) dan pilin A diregulasi bersama toxin (*tcpA*) dari *V. cholerae* telah diterokai dalam kajian ini. Kajian sebelum ini telah menunjukkan keupayaan peranan konstruk vaksin dwisistron *in vitro* melalui kejayaan pengekspresan dalam sel COS-7. Walau bagaimanapun, penilaian terhadap keimunogenan vaksin tersebut belum dilakukan secara *in vivo*. Oleh itu, objektif utama kajian ini adalah untuk menilai potensi *in vivo* vaksin mikropengkapsulan DNA plasmid (pDNA) dwisitronik terhadap jangkitan taun menggunakan model *Removable Intestinal Tie-Adult Rabbit Diarrhea* (RITARD). Bagi memastikan penyampaian melalui oral berjaya, pengkapsulan vaksin menggunakan mikrosfera alginat dihasilkan melalui pengemulsian air-dalam-minyak dengan purata keberkesanan pengkapsulan 88.6% dan selanjutnya, ia disahkan melalui ciri-ciri fizikal dan tingkah laku mikrosfera tersebut. Penyampaian pDNA yang berjaya juga dilihat dengan menggunakan simulasi keadaan pH gastrousus. Kemudian, penyampaian vaksin kapsul alginat melalui oral ini dilakukan secara *in vivo* terhadap arnab *New Zealand White* (NZW) berusia tujuh minggu. Lima belas ekor arnab betina digunakan dalam kajian ini dan dibahagikan kepada lima kumpulan: (1) Kawalan olahan jangkitan sebenar (kawalan negatif) (2) Mikrosfera alginat kosong (tidak divaksin); Mikrosfera alginat merangkumi (3) pVAX-*ctxB*; (4) pVAX-*tcpA*; dan (5) pVAX-*ctxB*-*tcpA*. Selain daripada kumpulan 1, kumpulan uji lain dicabar dengan

V. cholerae dari serotip Ogawa dan biotip El Tor, dua minggu setelah vaksinasi, dengan menggunakan model RITARD. Haiwan tersebut dipantau bagi gejala taun selama lima hari selepas pembedahan dan kultur swab rektum dari kumpulan 5 (konstruk vaksin dwisistronik) menunjukkan hasil negatif untuk organisma *V. cholerae*. Sebaliknya, jangkitan kolera adalah tidak ketara pada kumpulan vaksin dwisistronik kawalan berdasarkan pemeriksaan kasar dalam analisis bedah siasat dan histologi. Selain itu, tahap ekspresi *cytokines* (TNF-α, IFN-γ, IL-10 dan IL-6) dinilai menggunakan tindak balas berantai polimerase kuantitatif (qPCR) lanjutan daripada imunisasi dan jangkitan. Kumpulan dwisitronik menunjukkan peningkatan tahap IFN-γ dan IL-10 12 hari selepas vaksinasi, walaupun tidak signifikan, menunjukkan kebarangkalian pengaktifan respons *T-helper* 1 dan 2. Namun, tahap kesemua *cytokines* tidak berubah selepas jangkitan. Ringkasnya, alginat yang merangkumi dwisistronik pVAX-*ctxB*-*tcpA* menggambarkan potensi untuk mendorong tindak balas imun hos terhadap jangkitan taun, walaupun beberapa penambahbaikan dan analisis komprehensif perlu dilaksanakan untuk menilai peranannya sebagai vaksin DNA.

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I certify that a Thesis Examination Committee has met on 13 April 2021 to conduct the final examination of Najwa binti Ahmad Zamri on her thesis entitled "*In Vivo* Evaluation of Microencapsulated Bicistronic Plasmid DNA Vaccine against *Vibrio cholerae*" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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This is to confirm that:

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Signature:	
Name of Member of Supervisory Committee:	Prof. Madya Dr. Loqman Mohamad Yusof

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ALG	alginate
APC	antigen-presenting cell
cAMP	intracellular cyclic adenosine monophosphate
CD4+ /CD8+	cluster designation 4/8
cDNA	complimentary deoxyribonucleic acid
CFTR	cystic fibrosis transmembrane conductance regulator
CFU	colony forming units
cP	centipoise (unit)
CT	cholera toxin
CTA	cholera enterotoxin A subunit protein
CTB	cholera enterotoxin B subunit protein
CTL	cytotoxic T cell
ctxB	cholera toxin B subunit gene
DC	dendritic cell
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
EE	encapsulation efficiency
en [en(_)]	encapsulated
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GHRH	growth hormone-releasing hormone
GI	gastrointestinal tract
GM1	monosialotetrahexosylganglioside
GRAS	generally referred as safe
HCl	hydrochloric acid

IACUC	Institutional animal care and use committee
IEC	intestinal epithelial cell
IFN	interferon
IL	interleukin
LB	Luria Bertani
LPS	lipopolysaccharide
M cell	microfold cell
MHC	major histocompatibility complex
mRNA	messenger ribonucleic acid
mS	millisiemens (S. I. unit)
N	normality (S. I. unit)
NK	natural killer cell
NZW	New Zealand white rabbits
O. D.	Optical density
OCV	Oral cholera vaccine
PAMP	pathogen-associated molecular pattern
PBS	phosphate buffered saline
PCR	Polymerase chain reaction
pDNA	plasmid deoxyribonucleic acid
pH	potential hydrogen
PRR	pattern recognition receptor
rBS	cystic fibrosis transmembrane conductance regulator
RITARD	removable intestinal tie-adult rabbit diarrheal model
RNA	ribonucleic acid
rpm	revolutions per minute
SEM	standard error of mean

TCBS	thiosulfate citrate bile sucrose agar
TCP	toxin coregulated pilin
tcpA	toxin coregulated pilin A gene
Th1/Th2	T-helper 1 / T-helper 2
TLR	toll-like receptor
TNF-α	tumor necrosis factor - α
V	Volts (S. I. unit)
W/O	water-in-oil
w/v	weight per volume
WC	whole-cell
WHO	World Health Organization
Z-average	Cumulant mean

CHAPTER 1

INTRODUCTION

Cholera remains a public health burden particularly in areas with sanitation issues, lack of access to clean water or regions at risk of epidemic outbreaks. The fact that this disease is dependent on seasonal and environmental factors makes it to be classified as an 'emerging and re-emerging infection' in Africa, Asia and India (Mandal *et al*., 2011). In recent years, large-scale outbreaks of cholera were identified in Haiti and Yemen, where these countries were affected by environmental disaster and war crisis that disrupted water supplies, leading to contamination by the cholera agent. The World Health Organization (WHO) revealed that the reported cases of cholera are estimated to be three to five million cases occurring annually worldwide and the official report is believed to represent only 5-10% of the actual numerical figure globally (Ali *et al*., 2012). In addition, approximately 100 000 to 120 000 mortalities are estimated out of the reported cholera cases, reflecting the threat of the infection to society (Ali *et al*., 2012).

Cholera is a widely known diarrheal disease caused by a toxigenic Gram-negative bacterium known as *Vibrio cholerae* (*V. cholerae*), which is acquired through consumption of food or water contaminated by this microorganism (Almagro-Moreno & Taylor, 2013). Mandal *et al.* (2011) mentioned that among more than 200 serogroups of *V. cholerae* identified, only O1 and O139 serogroups cause epidemic cholera. These serogroups carry important virulence factors such as cholera toxin (CT) and toxin-coregulated pilus (TCP) that cause manifestation of cholera infection. In the worst scenario, cholera infection can cause a massive loss of body fluid resulting in severe dehydration or even death within hours in the absence of proper treatment (Sack *et al*., 2004).

Because of the threat of cholera to public health, several treatments and control measures have been introduced to reduce the burden. The current treatments to this infection include rehydration and antibiotic therapy (Harris *et al*., 2012). However, the widespread use of the latter treatment can lead to emergence of antibiotic resistance property of *V. cholerae* strains, making the microorganisms to be more aggressive and resistant to multiple types of drug (Bhattacharya *et al*., 2014). Besides that, the treatments serve as response initiatives that reduce the clinical symptoms of cholera. Thus, to control the prevalence of the disease in a whole population, a preventive approach such as inclusion of oral cholera vaccines (OCVs) to the public can be done.

The current control measure of the infection as promoted by WHO is by whole-cell oral cholera vaccination (OCV) using killed *V. cholerae* which has been proven to be efficacious since 1986, according to Clemens *et al.* (1986). However, the OCVs possess some drawbacks in terms of storage condition and dosage requirement which indirectly complicate the handling and management

processes. Firstly, the vaccines are registered to be kept cold before they are administered to individuals, which causes extra handling procedure of the vaccines. Secondly, there are concerns on the use of killed microorganisms as vaccines, especially in susceptible group of individuals such as pregnant women. Thirdly, the licensed protocol for the current OCVs requires two doses of the vaccines to be given to individuals in two weeks apart (Azman *et al*., 2015). The two-dose procedure makes it challenging to ensure everyone at risk of infection receives the appropriate amount of vaccine besides to assure the adequacy and availability of the vaccine stocks especially during outbreaks (Azman *et al*., 2015). Hence, there is a need for improvement of the vaccine strategy to establish a practical and efficient preventive measure that can reduce the incidence of cholera.

These constraints faced by the current OCVs can be potentially reduced with the technology of third generation vaccines, known as DNA vaccine. This is because the vaccine is easier to transport as it does not require a cold chain storage, hence, it is more stable and less expensive compared to conventional strategy (Abdo Hasson *et al*., 2015). Apart from the cost perspective, DNA vaccine is also safer since it consists of plasmid, which is a kind of non-replicating entity which in turn reduces the risk of reversion to a disease-causing state or secondary infection (Ferraro *et al*., 2011). Indeed, DNA vaccine has shown its potential as a promising approach in vaccine technology within the past decade based on several studies that demonstrated the success of DNA vaccine especially through licensure of DNA plasmid products for animal use. For instance, DNA-based therapy has been used for the treatment of hematopoeitic necrosis virus in salmon (Garver *et al*., 2005) and melanoma in dogs (Bergman *et al*., 2006). Besides that, it is also used as a growth hormone-releasing hormone (GHRH) gene therapy for swine (Draghia-Akli, 2015).

As for cholera, several studies have suggested the successful construction of DNA vaccine against this infection. One of the studies was by Syahril *et al.* (2002) in which the cholera vaccine was developed through insertion of *ctxB* genes of *Vibrio cholerae* into a mammalian expression plasmid DNA vector (pVax). In another study by Nograles (2013), single-gene insert of DNA vaccines comprising of *ctxB* and *tcpA* genes from *V. cholerae* were successfully constructed and tested *in vitro* and showed ability to trigger immune response *in vivo*. Hence, these led to the idea of constructing a bicistronic DNA vaccine that involves the integration of both *ctxB* and *tcpA* genes in a single plasmid in this project.

The application of a bicistronic DNA vaccine is also inciting the interest in the area of vaccinology. This new strategy involving integration of multiple antigens into a single array of plasmid is a useful tool to improve the efficacy of DNA vaccine formulation (Schirmbeck *et al*., 2000; Chinnasamy *et al*., 2006). This is because immunisation involving several proteins have provided solid protective immunity against leprosy in mice, suggesting the significance of multicistronic expression in eliciting immune responses (Gelber *et al*., 1994). A study by Cui *et al*. (2013) also supports the hypothesis, in which they proved that bicistronic

plasmid vaccine has the ability to induce immune responses against rotavirus infection in humoral, mucosal and cellular levels. However, in regards to cholera, there is no reported study that focuses on the immunogenicity of bicistronic plasmid vaccine *in vivo*. Thus, this project aims to determine the immunogenic potential of bicistronic plasmid DNA vaccine against cholera infection *in vivo*.

Objectives of the study

The study aims to evaluate the *in vivo* potential of microencapsulated bicistronic plasmid DNA vaccine against *Vibrio cholerae*.

The specific objectives of the study are:

1. To formulate and evaluate encapsulation of bicistronic plasmid DNA vaccine against cholera for oral delivery in rabbits.
2. To examine disease manifestation of rabbits infected with *V. cholerae* after vaccination with the DNA plasmid construct.
3. To quantify cytokine mRNA level of rabbits vaccinated with bicistronic plasmid DNA vaccine against *Vibrio cholerae*.

REFERENCES

Abdo Hasson, S. S. A., Al-Busaidi, J. K. Z., & Sallam, T. A. (2015). The past, current and future trends in DNA vaccine immunisations. *Asian Pacific Journal of Tropical Biomedicine*, *5*(5), 344–353. https://doi.org/10.1016/S2221-1691(15)30366-X

Abubakar, A., Azman, A. S., Rumunu, J., Ciglenecki, I., Helderman, T., West, H., Lessler, J., Sack. D. A., Martin, S., Perea, W., Legros, D. & Luquero, F. J. (2015). The First Use of the Global Oral Cholera Vaccine Emergency Stockpile: Lessons from South Sudan. *PLoS Medicine*, *12*(11), 1–8. https://doi.org/10.1371/journal.pmed.1001901

Akira, S., Uematsu, S., & Takeuchi, O. (2006). Pathogen Recognition and Innate Immunity. *Cell*, *124*(4), 783–801. https://doi.org/10.1016/j.cell.2006.02.015

Alam, M., Hasan, N. A., Sultana, M., Nair, G. B., Sadique, A., Faruque, A. S. G., Endtz, H. P., Sack, R. B., Huq, A., Colwell, R. R., Izumiya, H., Morita, M., Watanabe, H. & Cravioto, A. (2010). Diagnostic limitations to accurate diagnosis of cholera. *Journal of Clinical Microbiology*, *48*(11), 3918–3922. https://doi.org/10.1128/JCM.00616-10

Alam, M., Sultana, M., Nair, G. B., Sack, R. B., Sack, D. A., Siddique, A. K., Ali, A., Huq, A. & Colwell, R. R. (2006). Toxigenic *Vibrio cholerae* in the Aquatic Environment of Mathbaria, Bangladesh. *Applied and Environmental Microbiologytal Microbiology*, *72*(4), 2849–2855. https://doi.org/10.1128/AEM.72.4.2849

Alexakis, T., Boadi, D. K., Quong, D., Groboillot, A., Poncelet, D., & Neufeld, R. J. (1995). Microencapsulation of DNA within alginate microspheres and crosslinked chitosan membranes for *in vivo* application. *Applied Biochemistry and Biotechnology*, *50*(1), 93–106. https://doi.org/10.1007/BF02788043

Ali, M., Lopez, A. L., You, Y. A., Kim, Y. E., Sah, B., Maskery, B., & Clemens, J. (2012). The global burden of cholera. *Bulletin of the World Health Organization*, *90*(3), 209-218A. https://doi.org/10.2471/BLT.11.093427

Ali, M., Emch, M., Park, J. K., Yunus, M., & Clemens, J. (2011). Natural Cholera Infection–Derived Immunity in an Endemic Setting. *The Journal of Infectious Diseases*, *204*(6), 912–918. https://doi.org/10.1093/infdis/jir416

Ali, M., Nelson, A. R., Lopez, A. L., & Sack, D. A. (2015). Updated Global Burden of Cholera in Endemic Countries. *PLoS Neglected Tropical Diseases*, *9*(6), 1–13. https://doi.org/10.1371/journal.pntd.0003832

Almagro-Moreno, S., & Taylor, R. K. (2013). Cholera: Environmental Reservoirs and Impact on Disease Transmission. *Microbiology Spectrum*, *1*(2), 1–19. https://doi.org/10.1128/microbiolspec.OH-0003-2012

Attridge, S. R., Manning, P. A., Holmgren, J., & Jonson, G. (1996). Relative Significance of Mannose-Sensitive Hemagglutinin and Toxin-Coregulated Pili in Colonization of Infant Mice by *Vibrio cholerae* El Tor. *Infection and Immunity*, *64*(8), 3369–3373.

Azman, A. S., Luquero, F. J., Ciglenecki, I., Grais, R. F., Sack, D. A., & Lessler, J. (2015). The Impact of a One-Dose versus Two-Dose Oral Cholera Vaccine Regimen in Outbreak Settings: A Modeling Study. *PLoS Medicine*, *12*(8), 1–18. https://doi.org/10.1371/journal.pmed.1001867

Baker-Austin, C., Oliver, J. D., Alam, M., Ali, A., Waldor, M. K., Qadri, F., & Martinez-Urtaza, J. (2018). *Vibrio* spp. infections. *Nature Reviews Disease Primers*, *4*(8), 1–19. https://doi.org/10.1038/s41572-018-0005-8

Bandyopadhaya, A., Sarkar, M., & Chaudhuri, K. (2007). Transcriptional upregulation of inflammatory cytokines in human intestinal epithelial cells following *Vibrio cholerae* infection. *The FEBS Journal*, *274*, 4631–4642. https://doi.org/10.1111/j.1742-4658.2007.05991.x

Bari, W., Song, Y., & Yoon, S. S. (2011). Suppressed Induction of Proinflammatory Cytokines by a Unique Metabolite Produced by *Vibrio cholerae* O1 El Tor Biotype in Cultured Host Cells. *Infection and Immunity*, *79*(8), 3149–3158. https://doi.org/10.1128/IAI.01237-10

Barouch, D. H., Mckay, P. F., Sumida, S. M., Santra, S., Jackson, S. S., Gorgone, D. A., Lifton, M. A., Chakrabarti, B. K., Xu, L., Nabel, G. J., & Letvin, N. L. (2003). Plasmid Chemokines and Colony-Stimulating Factors Enhance the Immunogenicity of DNA Priming-Viral Vector Boosting Human Immunodeficiency Virus Type 1 Vaccines. *Journal of Virology*, *77*(16), 8729–8735. https://doi.org/10.1128/JVI.77.16.8729

Bergman, P. J., Camps-Palau, M. A., McKnight, J. A., Leibman, N. F., Craft, D. M., Leung, C., Liao, J., Riviere, I., Sadelain, M., Hohenhaus, A. E., Gregor, P., Houghton, A. N., Perales, M. A., & Wolchok, J. D. (2006). Development of a xenogeneic DNA vaccine program for canine malignant melanoma at the Animal Medical Center. *Vaccine*, *24*(21), 4582–4585. https://doi.org/10.1016/j.vaccine.2005.08.027

Bhakta, G., Mitra, S., & Amarnath, M. (2005). DNA encapsulated magnesium and manganous phosphate nanoparticles: potential non-viral vectors for gene delivery. *Biomaterials*, *26*(14), 2157–2163. https://doi.org/10.1016/j.biomaterials.2004.06.039

Bhattacharya, M. K., Kanungo, S., Ramamurthy, T., Rajendran, K., Sinha, A., Bhattacharya, A., & Sharma Sarkar, B. (2014). Comparison between Single Dose Azithromycin and Six Doses, 3 Day Norfloxacin for Treatment of Cholera in Adult. *International Journal of Biomedical Science*, *10*(4), 248–251.

Bhowmick, T. S., Koley, H., Das, M., Saha, D. R., & Sarkar, B. L. (2009). Pathogenic potential of vibriophages against an experimental infection with *Vibrio cholerae* O1 in the RITARD model. *International Journal of Antimicrobial Agents*, *33*(6), 569–573. https://doi.org/10.1016/j.ijantimicag.2008.10.034

Bhuiyan, T. R., Lundin, S. B., Khan, A. I., Lundgren, A., Harris, J. B., Calderwood, S. B., & Qadri, F. (2009). Cholera Caused by *Vibrio cholerae* O1 Induces T-Cell Responses in the Circulation. *Infection and Immunity*, *77*(5), 1888–1893. https://doi.org/10.1128/IAI.01101-08

Bishop, A. L., Patimalla, B., & Camilli, A. (2014). *Vibrio cholerae*-Induced Inflammation in the Neonatal Mouse Cholera Model. *Infection and Immunity*, *82*(6), 2434–2447. https://doi.org/10.1128/IAI.00054-14

Bowersock, T. L., HogenEsch, H., Suckow, M., Guimond, P., Martin, S., Borie, D., Torregrosa, S., Park, H. & Park, K. (1999). Oral vaccination of animals with antigens encapsulated in alginate microspheres. *Vaccine*, *17*, 1804–1811.

Butler, S. M., & Camilli, A. (2005). Going against the grain: chemotaxis and infection in *Vibrio cholerae*. *Nature Reviews Microbiology*, *3*(8), 611–620. https://doi.org/10.1038/nrmicro1207.GOING

Butterton, J. R., Boyko, S. A., & Calderwood, S. B. (1993). Use of the *Vibrio cholerae* irgA gene as a locus for insertion and expression of heterologous antigens in cholera vaccine strains. *Vaccine*, *11*(13), 1327–1335.

Butterton, J. R., Ryan, E. T., & Shahin, R. A. (1996). Development of a Germfree Mouse Model of *Vibrio cholerae* Infection. *Infection and Immunity*, *64*(10), 4373–4377.

Caetano, L. A., Almeida, A. J., & Gonçalves, L. M. D. (2016). Effect of Experimental Parameters on Alginate/Chitosan Microparticles for BCG Encapsulation. *Marine Drugs*, *14*(5), 1–30. https://doi.org/10.3390/md14050090

Chan, L. W., Ching, A. L., Liew, C. V., & Heng, P. W. S. (2007). Mechanistic Study on Hydration and Drug Release Behavior of Sodium Alginate Compacts. *Drug Development and Industrial Pharmacy*, *33*(6), 667–676. https://doi.org/10.1080/03639040600943814

Chen, S., Wu, Y., Mi, F., Lin, Y., Yu, L., & Sung, H. (2004). A novel pH-sensitive hydrogel composed of N, O-carboxymethyl chitosan and alginate cross-linked by genipin for protein drug delivery. *Journal of Controlled Release*, *96*(2), 285–300. https://doi.org/10.1016/j.jconrel.2004.02.002

Chinnasamy, D., Milsom, M. D., Shaffer, J., Neuenfeldt, J., Shaaban, A. F., Margison, G. P., Fairbairn, L. J., & Chinnasamy, N. (2006). Multicistronic lentiviral vectors containing the FMDV 2A cleavage factor demonstrate robust expression of encoded genes at limiting MOI. *Virology Journal*, *3*(14), 1–16. https://doi.org/10.1186/1743-422X-3-14

Cho, Y., Yi, H., Lee, J. H., Kim, D. W., & Chun, J. (2010). Genomic evolution of *Vibrio cholerae*. *Current Opinion in Microbiology*, *13*(5), 646–651. https://doi.org/10.1016/j.mib.2010.08.007

Clemens, J. D., Nair, G. B., Ahmed, T., Qadri, F., & Holmgren, J. (2017). Cholera. *The Lancet*, *390*, 1539–1549.

Clemens, J. D., Sack, D. A., Harris, J. R., Chakraborty, J., Khan, M. R., Stanton, B. F., Kay, B. A., Khan, M. U., Yunus, M., & Atkinson, W. (1986). Field trial of oral cholera vaccines in Bangladesh. *The Lancet*, *2*(8499), 124-127. https://doi.org/10.1016/S0140-6736(86)91944-6

Clemens, J. D., Sack, D. A., Harris, J. R., van Loon, F., Chakraborty, J., Ahmed, F., Rao, M. R., Khan, M. R., Yunus, M., Huda, N., Stanton, B. F., Kay, B. A., Walter, S., Eeckels, R., Svennerholm, A. M. & Holmgren, J. (1990). Field trial of oral cholera vaccines in Bangladesh: results from three-year follow-up. *The Lancet*, *335*(8684), 270–273.

Colwell, R. R., & Huq, A. (1970). Environmental Reservoir of *Vibrio cholerae*. The Causative Agent of Cholera. *Annals of the New York Academy of Sciences*, *740*, 44–54.

Colwell, Rita R, Huq, A., Islam, M. S., Aziz, K. M. A., Yunus, M., Khan, N. H., Mahmud, A., Sack, R. B., Nair, G. B., Chakraborty, J., Sack, D. A. & Russek-Cohen, E. (2003). Reduction of cholera in Bangladeshi villages by simple filtration. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(3), 1051–1055.

Conner, J. G., Teschler, J. K., Jones, C. J., & Yildiz, F. H. (2017). Staying alive: *Vibrio cholerae*'s cycle of environmental survival, transmission, and dissemination. *Microbiology Spectrum*, *4*(2), 1–48. https://doi.org/10.1128/microbiolspec.VMBF-0015-2015.Staying

Cray, W. C., Tokunaga, E., & Pierce, N. F. (1983). Successful Colonization and Immunization of Adult Rabbits by Oral Inoculation with *Vibrio cholerae* O1. *Infection and Immunity*, *41*(2), 735–741.

Crean, T. I., John, M., Calderwood, S. B., Ryan, E. T., Crean, T. I., John, M., & Calderwood, S. B. (2000). Optimizing the Germfree Mouse Model for *In Vivo* Evaluation of Oral *Vibrio cholerae* Vaccine and Vector Strains. *Infection and Immunity*, *68*(2), 977–981. https://doi.org/10.1128/IAI.68.2.977-981.2000

Cui, T., Xiong, J., Wang, Y., Wen, X., Huang, X., Huang, Y., Ma, X., Hu, Z., Zhao, Q., & Cao, S. (2013). Construction of an artificial recombinant bicistronic plasmid DNA vaccine against porcine rotavirus. *Onderstepoort Journal of Veterinary Research*, *80*(1), 1–8. https://doi.org/10.4102/ojvr.v80i1.498

Czerkinsky, C., Svennerholm, A., Quiding, M., Jonsson, R., & Holmgren, J. (1991). Antibody-Producing Cells in Peripheral Blood and Salivary Glands after Oral Cholera Vaccination of Humans. *Infection and Immunity*, *59*(3), 996–1001.

d'Ayala, G. G., Malinconico, M., & Laurienzo, P. (2008). Marine Derived Polysaccharides for Biomedical Applications: Chemical Modification Approaches. *Molecules*, *13*(9), 2069–2106. https://doi.org/10.3390/molecules13092069

Davis, J. A., & Banks, R. E. (1991). Modification to the RITARD Surgical Model. *Journal of Investigative Surgery*, *4*, 499–504.

De Magny, G. C., Mozumder, P. K., Grim, C. J., Hasan, N. A., Naser, M. N., Alam, M., Sack, R. B., Huq, A. & Colwell, R. R. (2011). Role of Zooplankton Diversity in *Vibrio cholerae* Population Dynamics and in the Incidence of Cholera in the Bangladesh Sundarbans. *Applied and Environmental Microbiology*, *77*(17), 6125–6132. https://doi.org/10.1128/AEM.01472-10

Desai, M. P., Labhasetwar, V., Amidon, G. L., & Levy, R. J. (1996). Gastrointestinal uptake of biodegradable microparticles: effect of particle size. *Pharmaceutical Research*, *13*(12), 1838–1845.

Dobakhti, F., Rahimi, F., Dehpour, A. R., Taghikhani, M., Ajdary, S., Rafiei, S., & Rafiee-Tehrani, M. (2006). Stabilizing effects of calcium alginate microspheres on mycobacterium bovis BCG intended for oral vaccination. *Journal of Microencapsulation*, *23*(8), 844–854. https://doi.org/10.1080/02652040601034013

Doria-rose, N. A., & Haigwood, N. L. (2003). DNA vaccine strategies : candidates for immune modulation and immunization regimens. *Methods*, *31*(3), 207–216. https://doi.org/10.1016/S1046-2023(03)00135-X

Dos Santos, L. L. (2017). *Natural Polymeric Biomaterials: Processing and Properties*. https://doi.org/10.1016/B978-0-12-803581-8.02253-0

Draghia-Akli, R., Ellis, K. M., Hill, L. A., Malone, P. B., & Fiorotto, M. L. (2015). High-efficiency growth hormone releasing hormone plasmid vector administration into skeletal muscle mediated by electroporation in pigs. *The FASEB Journal*, *17*(3), 526–528. https://doi.org/10.1096/fj.02-0671fje

Eko, F. O., Schukovskaya, T., Lotzmanova, E. Y., Firstova, V. V, Kutyrev, V. V, Igietseme, J. U., & Lubitz, W. (2003). Evaluation of the protective efficacy of *Vibrio cholerae* ghost (VCG) candidate vaccines in rabbits. *Vaccine*, *21*(25–26), 3663–3674. https://doi.org/10.1016/S0264-410X(03)00388-8

Espino, A. M., & Rivera, F. (2010). Quantitation of cytokine mRNA by real-time RT-PCR during a vaccination trial in a rabbit model of fascioliasis. *Veterinary Parasitology*, *169*(1–2), 82–92. https://doi.org/10.1016/j.vetpar.2009.12.018.

Falkard, B., Charles, R. C., Matias, W. R., Mayo-smith, L. M., Jerome, J. G., Offord, E. S., Xu, P., Kovac, P., Ryan, E. T., Qadri, F., Franke, M. F., Ivers, L. C. & Harris, J. B. (2019). Bivalent oral cholera vaccination induces a memory B cell response to the *V. cholerae* O1-polysaccharide antigen in Haitian adults. *PLoS Neglected Tropical Diseases*, *13*(1), 1–14.

Farris, E., Brown, D. M., Ramer-tait, A. E., & Pannier, A. K. (2016). Micro- and nanoparticulates for DNA vaccine delivery. *Experimental Biology and Medicine*, *241*, 919–929. https://doi.org/10.1177/1535370216643771

Faruque, A. S. G., Fuchs, G. J., & Albert, M. J. (1996). Changing epidemiology of cholera due to *Vibrio cholerae* O1 and O139 Bengal in Dhaka, Bangladesh. *Epidemiology and Infection*, *116*(3), 275–278.

Faruque, S. M., Biswas, K., Udden, S. M. N., Ahmad, Q. S., Sack, D. A., Nair, G. B., & Mekalanos, J. J. (2006). Transmissibility of cholera: *In vivo*-formed biofilms and their relationship to infectivity and persistence in the environment. *Proceedings of the National Academy of Sciences*, *103*(16), 6350–6355. https://doi.org/10.1073/pnas.0601277103

Faruque, Shah M, Albert, M. J., & Mekalanos, J. J. (1998). Epidemiology, Genetics, and Ecology of Toxigenic *Vibrio cholerae*. *Microbiology and Molecular Biology Reviews*, *62*(4), 1301–1314.

Ferraro, B., Morrow, M. P., Hutnick, N. A., Shin, T. H., Lucke, C. E., & Weiner, D. B. (2011). Clinical Applications of DNA Vaccines: Current Progress. *Clinical Infectious Diseases*, *53*(3), 296–302. https://doi.org/10.1093/cid/cir334

Finkelstein, R. A., & LoSpalluto, J. J. (1969). Pathogenesis of Experimental

Cholera: Preparation and Isolation of Choleragen and Choleragenoid. *The Journal of Experimental Medicine*, *130*(1), 185–202

.

Formal, S. B., Kundel, D., Schneider, H., Kunev, N., & Sprinz, H. (1961). Studies with *Vibrio cholerae* in the ligated loop of the rabbit intestine. *British Journal of Experimental Pathology*, *42*, 504–510. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/13894076%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2082487

Fullner, K. J., Boucher, J. C., Hanes, M. A., Haines III, G. K., Meehan, B. M., Walchle, C., Sansonetti, P. J. & Mekalanos, J. J. (2002). The Contribution of Accessory Toxins of *Vibrio cholerae* O1 El Tor to the Proinflammatory Response in a Murine Pulmonary Cholera Model. *The Journal of Experimental Medicine*, *195*(11), 1455–1462. https://doi.org/10.1084/jem.20020318

Fundueanu, G., Constantin, M., Dalpiaz, A., Bortolotti, F., Cortesi, R., Ascenzi, P., & Menegatti, E. (2004). Preparation and characterization of starch/cyclodextrin bioadhesive microspheres as platform for nasal administration of Gabexate Mesylate (Foy®) in allergic rhinitis treatment. *Biomaterials*, *25*(1), 159–170. https://doi.org/10.1016/S0142-9612(03)00477-0ca

Fynan, E. F., Webster, R. G., Fullert, D. H., Haynes, J. R., Santoro, J. C., & Robinson, H. L. (1993). DNA vaccines: Protective immunizations by parenteral, mucosal, and gene-gun inoculations. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(24), 11478–11482.

Gabriel, S. E., Brigman, K. N., Koller, B. H., Boucher, R. C., Gabriel, S. E., Brigman, K. N., Koller, B. H., Boucher, R. C. & Stutts, M. J. (1994). Cystic Fibrosis Heterozygote Resistance to Cholera Toxin in the Cystic Fibrosis Mouse Model. *Science*, *266*(5182), 107–109.

Gagliardi, M. C., Sallusto, F., Marinaro, M., Langerkamp, A., Lanzavecchia, A., & Magistris, M. T. De. (2000). Cholera toxin induces maturation of human dendritic cells and licences them for Th2 priming. *European Journal of Immunology*, *30*(8), 2394–2403. https://doi.org/10.1002/1521-4141(2000)30:8<2394::AID-IMMU2394>3.0.CO;2-Y

Garsin, D. A., Sifri, C. D., Mylonakis, E., Qin, X., Singh, K. V, Murray, B. E., Calderwood, S. B. & Ausubel, F. M. (2001). A simple model host for identifying Gram-positive virulence factors. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(19), 10892–10897.

Garver, K. A., LaPatra, S. E., & Kurath, G. (2005). Efficacy of an infectious hematopoietic necrosis (IHN) virus DNA vaccine in Chinook

Oncorhynchus tshawytscha and sockeye *O. nerka* salmon. *Diseases of Aquatic Organisms*, *64*, 13–22. https://doi.org/10.3354/dao064013

Gelber, R. H., Mehra, V., Bloom, B., Murray, L. P., Siu, P., Tsang, M., & Brennan, P. J. (1994). Vaccination with pure *Mycobacterium leprae* proteins inhibits *M. leprae* multiplication in mouse footpads. *Infection and Immunity*, *62*(10), 4250–4255. https://doi.org/10.1128/iai.62.10.4250-4255.1994

Gerdts, V., Wilson, H. L., Meurens, F., Van Drunen Littel - Van Den Hurk, S., Wilson, D., Walker, S., Wheler, C., Townsend, H. & Potter, A. A. (2015). Large Animal Models for Vaccine Development and Testing. *Institute of Laboratory Animal Research Journal*, *56*(1), 53–62. https://doi.org/10.1093/ilar/ilv009

Giulietti, A., Overbergh, L., Valckx, D., Decallonne, B., Bouillon, R., & Mathieu, C. (2001). An Overview of Real-Time Quantitative PCR: Applications to Quantify Cytokine Gene Expression. *Methods*, *25*, 386–401. https://doi.org/10.1006/meth.2001.1261

Gombotz, W. R., & Wee, S. F. (1998). Protein release from alginate matrices. *Advanced Drug Delivery Reviews*, *31*(3), 267–285.

Gorse, G. J., McElratht, M. J., Matthews, T. J., Hsieh, R. H., Belshe, R. B., Corey, L., Frey, S. E., Kennedy, D. J., Walker, M. C. & Eibl, M. M. (1998). Modulation of immunologic responses to HIV-1MN recombinant gp160 vaccine by dose and schedule of administration. *Vaccine*, *16*(5), 493–506.

Hanes, D. E., Robl, M. G., & Schneider, C. M. (2001). New Zealand White Rabbit as a Nonsurgical Experimental Model for *Salmonella enterica* Gastroenteritis. *Infection and Immunity*, *69*(10), 6523–6526. https://doi.org/10.1128/IAI.69.10.6523

Hanes, J., Cleland, J. L., & Langer, R. (1997). New advances in microsphere-based single-dose vaccines. *Advanced Drug Delivery Reviews*, *28*(1), 97–119.

Harris, A. M., Bhuiyan, M. S., Chowdhury, F., Khan, A. I., Hossain, A., Kendall, E. A., Rahman, A., LaRocque, R. C., Wrammert, J., Ryan, E. T., Qadri, F., Calderwood, S. B. & Harris, J. B. (2009). Antigen-Specific Memory B-Cell Responses to *Vibrio cholerae* O1 Infection in Bangladesh. *Infection and Immunity*, *77*(9), 3850–3856. https://doi.org/10.1128/IAI.00369-09

Harris, J B, LaRocque, R. C., Qadri, F., Ryan, E. T., & Calderwood, S. B. (2012). Cholera. *Lancet*, *379*(9835), 2466–2476. https://doi.org/10.1016/S0140-6736(12)60436-X.Cholera

Harris, Jason B. (2018). Cholera: Immunity and Prospects in Vaccine

Development. *The Journal of Infectious Diseases*, *218*(S3), 141–146. https://doi.org/10.1093/infdis/jiy414

Herrington, D. A., Hall, R. H., Losonsky, G., Mekalanos, J. J., Taylor, R. K., & Levine, M. M. (1988). Toxin, Toxin-Coregulated Pili, and the toxR Regulon are Essential for *Vibrio cholerae* Pathogenesis in Humans. *Journal of Experimental Medicine*, *168*(4), 1487–1492.

Huang, K. S., Lin, Y. S., Yang, C. H., Tsai, C. W., & Hsu, M. Y. (2011). *In situ* synthesis of twin monodispersed alginate microparticles. *Soft Matter*, *7*(14), 6713–6718. https://doi.org/10.1039/c0sm01361g

Huq, A, Xu, B., Chowdhury, M. A. R., Islam, M. S., Montilla, R., & Colwell, R. R. (1996). A Simple Filtration Method To Remove Plankton-Associated *Vibrio cholerae* in Raw Water Supplies in Developing Countries. *Applied and Environmental Microbiology*, *62*(7), 2508–2512.

Huq, Anwar, Sack, R. B., Nizam, A., Longini, I. M., Nair, G. B., Ali, A., Morris Jr., J. G., Khan, M. N. H., Siddique, A. K., Yunus, M., Albert, M. J., Sack, D. A. & Colwell, R. R. (2005). Critical Factors Influencing the Occurrence of *Vibrio cholerae* in the Environment of Bangladesh. *Applied and Environmental Microbiology*, *71*(8), 4645–4654. https://doi.org/10.1128/AEM.71.8.4645

Janeway, C. A. (1992). The immune system evolved to discriminate infectious nonself from noninfectious self. *Immunology Today*, *13*(1), 11–16.

Jobling, M. G., Yang, Z. J., Kam, W. R., Lencer, W. I., & Holmes, R. K. (2012). A Single Native Ganglioside GM1-Binding Site Is Sufficient for Cholera Toxin To Bind to Cells and Complete the Intoxication Pathway. *MBio*, *3*(6), 1–9. https://doi.org/10.1128/mBio.00401-12

Johansson, M. E. V, Sjovall, H., & Hansson, G. C. (2013). The gastrointestinal mucus system in health and disease. *Nature Reviews Gastroenterology & Hepatology*, *10*(6), 352–361. https://doi.org/10.1038/nrgastro.2013.35.The

Kaper, J. B., Morris, J. G., & Levine, M. M. (1995). Cholera. *Clinical Microbiology Reviews*, *8*(1), 48–86.

Kawai, T., & Akira, S. (2007). TLR signaling. *Seminars in Immunology*, *19*(1), 24–32. https://doi.org/10.1016/j.smim.2006.12.004

Keitel, W. A., Treanor, J. J., Sahly, H. M. El, Evans, T. G., Kopper, S., Whitlow, V., Selinsky, C., Kaslow, D. C., Rolland, A., Smith, L. R. & Lalor, P. A. (2009). Evaluation of a plasmid DNA-based anthrax vaccine in rabbits, nonhuman primates and healthy adults. *Human Vaccines*, *5*(8), 536–544. https://doi.org/10.4161/hv.5.8.8725

Kenney, R. T., & Edelman, R. (2003). Survey of human-use adjuvants. *Expert Review of Vaccines*, *2*(2), 167–188.

Kidane, A., Guimond, P., Ju, T. R., Sanchez, M., Gibson, J., North, A., HogenEsch, H. & Bowersock, T. L. (2002). Effects of cellulose derivatives and poly(ethylene oxide)– poly(propylene oxide) tri-block copolymers (Pluronic surfactants) on the properties of alginate based microspheres and their interactions with phagocytic cells. *Journal of Controlled Release*, *85*, 181–189.

Klose, K. E. (2000). The suckling mouse model of cholera. *Trends in Microbiology*, *8*(4), 189–191. https://doi.org/10.1016/S0966-842X(00)01721-2

Koch, R. (1884). An Address on Cholera and Its Bacillus. *The British Medical Journal*, *2*(1235), 403–407.

Kuchta, A., Rahman, T., Sennott, E. L., Bhuyian, T. R., Uddin, T., Rashu, R., Chowdury, F., Kahn, A. I., Arifuzzaman, M., Weil, A. A., Podolsky, M., LaRocque, R. C., Ryan, E. T., Calderwood, S. B. & Harris, J. B. (2011). *Vibrio cholerae* O1 Infection Induces Proinflammatory CD4+ T-Cell Responses in Blood and Intestinal Mucosa of Infected Humans. *Clinical and Vaccine Immunology*, *18*(8), 1371–1377. https://doi.org/10.1128/CVI.05088-11

Kumru, O. S., Joshi, S. B., Smith, D. E., Middaugh, C. R., Prusik, T., & Volkin, D. B. (2014). Vaccine instability in the cold chain: Mechanisms, analysis and formulation strategies. *Biologicals*, *42*(5), 237–259. https://doi.org/10.1016/j.biologicals.2014.05.007

Langer, B., Renner, M., Scherer, J., Schüle, S., & Cichutek, K. (2013). Safety Assessment of Biolistic DNA Vaccination. *Biolistic DNA Delivery*, *940*, 371–388. https://doi.org/10.1007/978-1-62703-110-3

Larhed, A. W., Artursson, P., Grasjo, J., & Bjork, E. (1997). Diffusion of Drugs in Native and Purified Gastrointestinal Mucus. *Journal of Pharmaceutical Sciences*, *86*(6), 660–665.

Lavelle, E. C., McNeela, E., Armstrong, M. E., Leavy, O., Higgins, S. C., & Mills, K. H. G. (2003). Cholera Toxin Promotes the Induction of Regulatory T Cells Specific for Bystander Antigens by Modulating Dendritic Cell Activation. *The Journal of Immunology*, *171*(5), 2384–2392. https://doi.org/10.4049/jimmunol.171.5.2384

Lee, K. Y., & Mooney, D. J. (2012). Alginate : properties and biomedical applications. *Progress in Polymer Science*, *37*(1), 106–126. https://doi.org/10.1016/j.progpolymsci.2011.06.003.Alginate

Leong, J., Lam, W., Ho, K., Voo, W., Lee, M. F.-X., Lim, H. P., Lim, S. L., Tey, B. T., Poncelet, D. & Chan, E. (2016). Advances in fabricating

spherical alginate hydrogels with controlled particle designs by ionotropic gelation as encapsulation systems. *Particuology*, *24*, 44–60. https://doi.org/10.1016/j.partic.2015.09.004

Levinson, D. R. (2012). *Vaccines for Children Program: Vulnerabilities in Vaccine Management*.

Li, M., Carpio, D. F., Zheng, Y., Bruzzo, P., Ouaaz, F., Medzhitov, R. M., & Beg, A. A. (2001). An Essential Role of the NF-κB/Toll-Like Receptor Pathway in Induction of Inflammatory and Tissue-Repair Gene Expression by Necrotic Cells. *The Journal of Immunology*, *166*(12), 7128–7135. https://doi.org/10.4049/jimmunol.166.12.7128

Liang, W., Wang, S., Yu, F., Zhang, L., Qi, G., Liu, Y., Gao, S. & Kan, B. (2003). Construction and Evaluation of a Safe, Live, Oral *Vibrio cholerae* Vaccine Candidate, IEM108. *Infection and Immunity*, *71*(10), 5498–5504. https://doi.org/10.1128/IAI.71.10.5498

Lindenbaum, J., Greenough, W. B., & Islam, M. R. (1967). Antibiotic therapy of cholera in children. *Bulletin of the World Health Organization*, *37*(4), 529–538.

López-Bravo, M., & Ardavin, C. (2008). *In Vivo* Induction of Immune Responses to Pathogens by Conventional Dendritic Cells. *Immunity*, *29*(3), 343–351. https://doi.org/10.1016/j.immuni.2008.08.008

Lopez, A. L., Dutta, S., Qadri, F., Sovann, L., Pandey, B. D., Hamzah, W. M., Memon, I., Iamsirithaworn, S., Anh, D. D., Chowdury, F., Heng, S., Kanungo, S., Mogasale, V., Sultan, A. & Ylade, M. (2020). Cholera in selected countries in Asia. *Vaccine*, *38*, A18–A24. https://doi.org/https://doi.org/10.1016/j.vaccine.2019.07.035

Loureiro, J., & Ploegh, H. L. (2006). Antigen Presentation and the Ubiquitin-Proteasome System in Host-Pathogen Interactions. *Advances in Immunology*, *92*, 225–305. https://doi.org/10.1016/S0065-2776(06)92006-9

Lozier, J. N., Yankaskas, J. R., Ramsey, W. J., Chen, L., Berschneider, H., & Morgan, R. A. (1997). Gut Epithelial Cells as Targets for Gene Therapy of Hemophilia. *Human Gene Therapy*, *8*(12), 1481–1490. https://doi.org/https://doi.org/10.1089/hum.1997.8.12-1481

Lu, B., Wen, R., Yang, H., & He, Y. (2007). Sustained-release tablets of indomethacin-loaded microcapsules: Preparation, *in vitro* and *in vivo* characterization. *International Journal of Pharmaceutics*, *333*, 87–94. https://doi.org/10.1016/j.ijpharm.2006.10.002

Lund, F. E. (2009). Cytokine-producing B Lymphocytes - Key Regulators of Immunity. *Curent Opinion in Immunology*, *20*(3), 332–338.

Machado, A. H. E., Lundberg, D., Ribeiro, J., Veiga, F. J., Miguel, M. G., Bijorn, L., & Olsson, U. (2013). Encapsulation of DNA in Macroscopic and Nanosized Calcium Alginate Gel Particles. *Langmuir*, *29*(51), 15926–15935.

Mandal, S., Mandal, M. D., & Pal, N. K. (2011). Cholera: A great global concern. *Asian Pacific Journal of Tropical Medicine*, *4*(7), 573–580. https://doi.org/10.1016/S1995-7645(11)60149-1

Mansour, H. M., Sohn, M. J., Al-Ghananeem, A., & DeLuca, P. P. (2010). Materials for pharmaceutical dosage forms: Molecular pharmaceutics and controlled release drug delivery aspects. *International Journal of Molecular Sciences*, *11*(9), 3298–3322. https://doi.org/10.3390/ijms11093298

Martinsen, A., Storro, I., & Skjak-Braek, G. (1992). Alginate as Immobilization Material: III. Diffusional Properties. *Biotechnology and Bioengineering*, *39*(2), 186–194.

Mathan, M. M., Chandy, G., & Mathan, V. I. (1995). Ultrastructural Changes in the Upper Small Intestinal Mucosa in Patients With Cholera. *Gastroenterology*, *109*(2), 422–430.

Matthias, D. M., Robertson, J., Garrison, M. M., Newland, S., & Nelson, C. (2007). Freezing temperatures in the vaccine cold chain: A systematic literature review. *Vaccine*, *25*(20), 3980–3986. https://doi.org/10.1016/j.vaccine.2007.02.052

Mayo-smith, L. M., Simon, J. K., Chen, W. H., Haney, D., Lock, M., Lyon, C. E., Calderwood, S. B., Kirkpatrick, B. D., Cohen, M., Levine, M. M., Gurwith, M. & Harris, J. B. (2017). The Live Attenuated Cholera Vaccine CVD 103-HgR Primes Responses to the Toxin-Coregulated Pilus Antigen TcpA in Subjects Challenged with Wild-Type *Vibrio cholerae*. *Clinical and Vaccine Immunology*, *24*(1), 1–10. https://doi.org/https://doi.org/10.1128/CVI.00470-16

McGee, D. W., Elson, C. O., & McGhee, J. R. (1993). Enhancing Effect of Cholera Toxin on Interleukin-6 Secretion by IEC-6 Intestinal Epithelial Cells: Mode of Action and Augmenting Effect of Inflammatory Cytokines. *Infection and Immunity*, *61*(11), 4637–4644.

Mena, A., Ioannou, X. P., Kessel, A. Van, Van Drunen Little-Van Den Hurk, S., Popowych, Y., Babiuk, L. A., & Godson, D. L. (2002). Th1/Th2 biasing effects of vaccination in cattle as determined by real-time PCR. *Journal of Immunological Methods*, *263*(1–2), 11–21.

Merrell, D. S., Butler, S. M., Qadri, F., Dolganov, N. A., Alam, A., Cohen, M. B., Calderwood, S. B., Schoolnik, G. K. & Camilli, A. (2002). Host-induced epidemic spread of cholera bacterium. *Nature*, *417*(6889), 642–645. https://doi.org/10.1038/nature00778.Host-induced

Millet, Y. A., Alvarez, D., Ringgaard, S., Andrian, U. H. Von, Davis, B. M., & Waldor, M. K. (2014). Insights into *Vibrio cholerae* Intestinal Colonization from Monitoring Fluorescently Labeled Bacteria. *PLoS Pathogens*, *10*(10), 1–14. https://doi.org/10.1371/journal.ppat.1004405

Mintz, E. D., & Guerrant, L. (2009). A Lion in Our Village - The Unconscionable Tragedyof Cholera in Africa. *The New England Journal of Medicine*, *360*(11), 1060–1063. https://doi.org/https://doi.org/10.1056/NEJMp0810559

Mir, F., Kaufmann, S. H. E., & Eddine, A. N. (2009). *A Multicistronic DNA Vaccine Induces Significant Protection against Tuberculosis in Mice and Offers Flexibility in the Expressed Antigen Repertoire □*. *16*(10), 1467–1475. https://doi.org/10.1128/CVI.00237-09

Miyazaki, Y., Yakou, S., & Takayama, K. (2004). Study on jelly fig extract as a potential hydrophilic matrix for controlled drug delivery. *International Journal of Pharmaceutics*, *287*(1–2), 39–46. https://doi.org/10.1016/j.ijpharm.2004.08.023

Mondal, M., Nag, D., Koley, H., Saha, D. R., & Chatterjee, N. S. (2014). The *Vibrio cholerae* Extracellular Chitinase ChiA2 Is Important for Survival and Pathogenesis in the Host Intestine. *PLoS ONE*, *9*(9), 1–12. https://doi.org/10.1371/journal.pone.0103119

Morel, P. A., & Turner, M. S. (2010). Designing the optimal vaccine: the importance of cytokines and dendritic cells. *Open Vaccine Journal*, *3*, 7–17. https://doi.org/10.2174/1875035401003010007

Morris Jr, J. G. (2003). *Cholera and Other Types of Vibriosis : A Story of Human Pandemics and Oysters on the Half Shell*. *21201*.

Moser, M., & Leo, O. (2010). Key concepts in immunology. *Vaccine*, *28*, 2–13. https://doi.org/10.1016/j.vaccine.2010.07.022

Mosley II, J. F., Smith, L. L., Brantley, P., Locke, D., & Como, M. (2017). Vaxchora: The First FDA-Approved Cholera Vaccination in the United States. *Pharmacy & Therapeutics*, *42*(10), 638–640.

Mukhopadhyay, A. K., Takeda, Y., & Nair, G. B. (2014). Cholera Outbreaks in the El Tor Biotype Era and the Impact of the New El Tor Variants. *Current Topics in Microbiology and Immunology*, *379*, 17–47. https://doi.org/10.1007/82

Mumper, R. J., Hoffman, A. S., Puolakkainenb, P. A., Bouchardb, L. S., & Gombotzb, W. R. (1994). Calcium-alginate beads for the oral delivery of transforming growth factor-B1 (TGF-B1) : stabilization of TGF-B1 by the addition of polyacrylic acid within acid-treated beads. *Journal of Controlled Release*, *30*(3), 241–251.

Nait Mohamed, F. A., & Laraba-djebari, F. (2016). Development and characterization of a new carrier for vaccine delivery based on calcium-alginate nanoparticles: Safe immunoprotective approach against scorpion envenoming. *Vaccine*, *34*(24), 2692–2699. https://doi.org/10.1016/j.vaccine.2016.04.035

Nelson, E. J., Chowdhury, A., Harris, J. B., Begum, Y. A., Chowdhury, F., Khan, A. I., LaRocque, R. C., Bishop, A. L., Ryan, E. T., Camilli, A., Qadri, F. & Calderwood, S. B. (2007). Complexity of rice-water stool from patients with *Vibrio cholerae* plays a role in the transmission of infectious diarrhea. *Proceedings of the National Academy of Sciences*, *104*(48), 19091–19096. https://doi.org/10.1073/pnas.0706352104

Nelson, E. J., Harris, J. B., Morris Jr, J. G., Calderwood, S. B., & Camilli, A. (2009). Cholera transmission: the host, pathogen and bacteriophage dynamics. *Nature Reviews Microbiology*, *7*(10), 1–24. https://doi.org/10.1038/nrmicro2204.Cholera

New, R. R. C. (2019). Formulation technologies for oral vaccines. *Clinical and Experimental Immunology*, *198*(2), 153–169. https://doi.org/10.1111/cei.13352

Newman, K. D., Elamanchili, P., Kwon, G. S., & Samuel, J. (2001). Uptake of poly (D, L-lactic-co-glycolic acid) microspheres by antigen-presenting cells *in vivo*. *Journal of Biomedical Materials Research*, *60*(3), 480–486. https://doi.org/10.1002/jbm.10019

Nielsen, A. T., Dolganov, N. A., Rasmussen, T., Otto, G., Miller, M. C., Felt, S. A., Torreilles, S. & Schoolnik, G. K. (2010). A Bistable Switch and Anatomical Site Control *Vibrio cholerae* Virulence Gene Expression in the Intestine. *PLoS Pathogens*, *6*(9), 1–23. https://doi.org/10.1371/journal.ppat.1001102

Nielsen, Alex Toftgaard, Dolganov, N. A., Otto, G., Miller, M. C., Wu, C. Y., & Schoolnik, G. K. (2006). RpoS Controls the *Vibrio cholerae* Mucosal Escape Response. *PLoS Pathogens*, *2*(10), 933–948. https://doi.org/10.1371/journal.ppat.0020109

Nograles, N., Abdullah, S., Shamsudin, M. N., Billa, N., & Rosli, R. (2012). Formation and characterization of pDNA-loaded alginate microspheres for oral administration in mice. *Journal of Bioscience and Bioengineering*, *113*(2), 133–140. https://doi.org/10.1016/j.jbiosc.2011.10.003

Nograles, N. H. Oral Immunization with Cholera DNA Vaccine Employing Microencapsulation Delivery System. PhD dissertation. Universiti Putra Malaysia, Serdang. January 2013.

Norris, D. A., & Sinko, P. J. (1996). Effect of Size, Surface Charge, and Hydrophobicity on the Translocation of Polystyrene Microspheres

Through Gastrointestinal Mucin. *Journal of Applied Polymer Science*, *63*(11), 1481–1492.

O'Hagan, D. T., & Rappuoli, R. (2006). Novel approaches to pediatric vaccine delivery. *Advanced Drug Delivery Reviews*, *58*, 29–51. https://doi.org/10.1016/j.addr.2005.12.002

O'Hagan, D. T., & Singh, M. (2004). Microparticles as vaccine adjuvants and delivery systems. *Novel Vaccination Strategies*, *2*(2), 147–171.

O'Neill, M. J., Bourre, L., Melgar, S., & O'Driscoll, C. M. (2011). Intestinal delivery of non-viral gene therapeutics: physiological barriers and preclinical models. *Drug Discovery Today*, *16*(5–6), 203–218. https://doi.org/10.1016/j.drudis.2011.01.003

Olivier, V., Salzman, N. H., & Satchell, K. J. F. (2007). Prolonged Colonization of Mice by *Vibrio cholerae* El Tor O1 Depends on Accessory Toxins. *Infection and Immunity*, *75*(10), 5043–5051. https://doi.org/10.1128/IAI.00508-07

Page, D. T., & Cudmore, S. (2001). Innovations in oral gene delivery: challenges and potentials. *Drug Discovery Today*, *6*(2), 92–101.

Panyam, J., Dali, M. M., Sahoo, S. K., Ma, W., Chakravarthi, S. S., Amidon, G. L., Levy, R. J. & Labhasetwar, V. (2003). Polymer degradation and *in vitro* release of a model protein from poly(D,L-lactide-co-glycolide) nano- and microparticles. *Journal of Controlled Release*, *92*(1–2), 173–187. https://doi.org/10.1016/S0168-3659(03)00328-6

Pastor, M., Esquisabel, A., Talavera, A., Ano, G., Fernández, S., Cedré, B., Infante, J. F., Callicó, A. & Pedraz, J. L. (2013). An approach to a cold chain free oral cholera vaccine : *in vitro* and *in vivo* characterization of *Vibrio cholerae* gastro-resistant microparticles. *International Journal of Pharmaceutics*, *448*, 247–258. https://doi.org/10.1016/j.ijpharm.2013.02.057

Patel, N., Lalwani, D., Gollmer, S., Injeti, E., Sari, Y., & Nesamony, J. (2016). Development and evaluation of a calcium alginate based oral ceftriaxone sodium formulation. *Progress in Biomaterials*, *5*(2), 117–133. https://doi.org/10.1007/s40204-016-0051-9

Pawar, S. N., & Edgar, K. J. (2012). Alginate derivatization: A review of chemistry, properties and applications. *Biomaterials*, *33*(11), 3279–3305. https://doi.org/10.1016/j.biomaterials.2012.01.007

Peppas, N. A., & Buri, P. A. (1985). Surface, Interfacial and Molecular Aspects of Polymer Bioadhesion on Soft Tissues. *Journal of Controlled Release*, *2*, 257–275.

Perumal, D. (2001). Microencapsulation of ibuprofen and Eudragit® RS 100

by the emulsion solvent diffusion technique. *International Journal of Pharmaceutics*, *218*(1–2), 1–11.

Plotkin, S. A. (2008). Correlates of Vaccine-Induced Immunity. *Clinical Infectious Diseases*, *47*(3), 401–409. https://doi.org/10.1086/589862

Pulendran, B., & Ahmed, R. (2012). Immunological mechanisms of vaccination. *Nature Immunology*, *12*(6), 509–517.

Randolph, G. J., Inaba, K., Robbiani, D. F., Steinman, R. M., & Muller, W. A. (1999). Differentiation of Phagocytic Monocytes into Lymph Node Dendritic Cells *In Vivo*. *Immunity*, *11*(6), 753–761.

Reed, S. G., Bertholet, S., Coler, R. N., & Friede, M. (2009). New horizons in adjuvants for vaccine development. *Trends in Immunology*, *30*(1), 23–32. https://doi.org/10.1016/j.it.2008.09.006

Rice-ficht, A. C., Arenas-gamboa, A. M., Kahl-McDonagh, M. M., & Ficht, T. A. (2010). Polymeric particles in vaccine delivery. *Current Opinion in Microbiology*, *13*, 106–112. https://doi.org/10.1016/j.mib.2009.12.001

Richie, E., Punjabi, N. H., Sidharta, Y., Peetosutan, K., Sukandar, M., Wasserman, S. S., Lesmana, M., Wangsasaputra, F., Pandam, S., Levine, M. M., O'Hanley, P., Cryz, S. J. & Simanjuntak, C. H. (2000). Efficacy trial of single-dose live oral cholera vaccine CVD 103-HgR in North Jakarta, Indonesia, a cholera-endemic area. *Vaccine*, *18*(22), 2399–2410.

Ritchie, J. M., Rui, H., Bronson, R. T., & Waldor, M. K. (2010). Back to the Future: Studying Cholera Pathogenesis Using Infant. *MBio*, *1*(1), 1–13. https://doi.org/10.1128/mBio.00047-10.Editor

Rodrigues, A. P., Hirsch, D., Figueiredo, H. C. P., Logato, P. V. R., & Moraes, A. M. (2006). Production and characterisation of alginate microparticles incorporating *Aeromonas hydrophila* designed for fish oral vaccination. *Process Biochemistry*, *41*, 638–643. https://doi.org/10.1016/j.procbio.2005.08.010

Rollenhagen, J. E., Kalsy, A., Cerda, F., John, M., Harris, J. B., LaRocque, R. C., Qadri, F., Calderwood, S. B., Taylor, R. K. & Ryan, E. T. (2006). Transcutaneous Immunization with Toxin-Coregulated Pilin A Induces Protective Immunity against *Vibrio cholerae* O1 El Tor Challenge in Mice. *Infection and Immunity*, *74*(10), 5834–5839. https://doi.org/10.1128/IAI.00438-06

Rong, J., Liang, M., Xuan, F., Sun, J., Zhao, L., Zhen, H., Tian, X., Liu, D., Zhang, Q., Peng, C., Yao, T., Li, F., Wang, X., Han Y. & Yu, W. (2015). Alginate-calcium microsphere loaded with thrombin : A new composite biomaterial for hemostatic embolization. *International Journal of Biological Macromolecules*, *75*, 479–488.

https://doi.org/http://dx.doi.org/10.1016/j.ijbiomac.2014.12.043

Roy, N., Barman, S., Ghosh, A., Pal, A., Chakraborty, K., Das, S. S., Saha, D. R., Yamasaki, S. & Koley, H. (2010). Immunogenicity and protective efficacy of *Vibrio cholerae* outer membrane vesicles in rabbit model. *FEMS Immunology & Medical Microbiology*, *60*(1), 18–27. https://doi.org/10.1111/j.1574-695X.2010.00692.x

Runft, D. L., Mitchell, K. C., Abuaita, B. H., Allen, J. P., Bajer, S., Ginsburg, K., Neely, M. N. & Withey, J. H. (2014). Zebrafish as a Natural Host Model for *Vibrio cholerae* Colonization and Transmission. *Applied and Environmental Microbiology*, *80*(5), 1710–1717. https://doi.org/10.1128/aem.03580-13

Russell, R. G., Tall, B. E. N. D., & Morris, J. G. (1992). Non-O1 *Vibrio cholerae* Intestinal Pathology and Invasion in the Removable Intestinal Tie Adult Rabbit Diarrhea Model. *Infection and Immunity*, *60*(2), 435–442.

Ryan, E. T., & Calderwood, S. B. (2000). Cholera Vaccines. *Clinical Infectious Diseases*, *31*(2), 561–565.

Ryan, E. T., & Calderwood, S. B. (2001). Cholera Vaccines. *Journal of Travel Medicine*, *8*(2), 82–91.

Sack, D. A., Sack, R. B., Nair, G. B., & Siddique, A. (2004). Cholera. *The Lancet*, *363*(9404), 223–233. https://doi.org/10.1016/S0140-6736(03)15328-7

Sallusto, F., Lanzavecchia, A., Araki, K., & Ahmed, R. (2013). From Vaccines to Memory and Back. *Immunity*, *33*(4), 451–463. https://doi.org/10.1016/j.immuni.2010.10.008.From

Sanchez, J., & Holmgren, J. (2008). Cholera toxin structure, gene regulation and pathophysiological and immunological aspects. *Cellular and Molecular Life Sciences*, *65*(9), 1347–1360. https://doi.org/10.1007/s00018-008-7496-5

Sarkar, M., Bhowmick, S., Casola, A., & Chaudhuri, K. (2012). Interleukin-8 gene regulation in epithelial cells by *Vibrio cholerae*: role of multiple promoter elements, adherence and motility of bacteria and host MAPKs. *The FEBS JournalFEBS Journal*, *279*(8), 1464–1473. https://doi.org/10.1111/j.1742-4658.2012.08539.x

Satitsri, S., Pongkorpsakol, P., Srimanote, P., Chatsudthipong, V., & Muanprasat, C. (2016). Pathophysiological mechanisms of diarrhea caused by the *Vibrio cholerae* O1 El Tor variant: an *in vivo* study in mice. *Virulence*, *7*(7), 789–805. https://doi.org/10.1080/21505594.2016.1192743

Schijns, V. E. J. C. (2002). Antigen delivery systems and immunostimulation. *Veterinary Immunology and Immunopathology*, *87*(3–4), 195–198.

Schirmbeck, R., Kampen, J. Von, Metzger, K., Wild, J., Grüner, B., Schleef, M., Kröger, A., Hauser, H., & Reimann, J. (2000). DNA-Based Vacination with Polycistronic Expression Plasmids. *Methods in Molecular Medicine*, *29*, 313–322. https://doi.org/10.1385/1-59259-688-6:313

Schrank, G. D., & Verwey, W. F. (1976). Distribution of cholera organisms in experimental *Vibrio cholerae* infections: proposed mechanisms of pathogenesis and antibacterial immunity. *Infection and Immunity*, *13*(1), 195–203.

Senderovich, Y., Izhaki, I., & Halpern, M. (2010). Fish as Reservoirs and Vectors of *Vibrio cholerae*. *PLoS ONE*, *5*(1), e8607. https://doi.org/10.1371/journal.pone.0008607

Shakya, A. K., & Nandakumar, K. S. (2012). Polymers as immunological adjuvants: An update on recent developments. *Journal of BioScience & Biotechnology*, *1*(3), 199–210.

Shalaby, K. S., Soliman, M. E., Casettari, L., Bonacucina, G., Cespi, M., Palmieri, G. F., Sammour, O. & El Shamy, A. A. (2014). Determination of factors controlling the particle size and entrapment efficiency of noscapine in PEG / PLA nanoparticles using artificial neural networks. *International Journal of Nanomedicine*, *9*(1), 4953–4964. https://doi.org/http://dx.doi.org/10.2147/IJN.S68737

Silva, A. J., Eko, F. O., & Benitez, J. A. (2009). Exploiting cholera vaccines as a versatile antigen delivery platform. *Biotechnology Letters*, *30*(4), 571–579. https://doi.org/10.1007/s10529-007-9594-0.Exploiting

Silva, T. M. J., Schleupner, M. A., Tacket, C. O., Steiner, T. E. D. S., Kaper, J. B., Edelman, R., & Guerrant, R. L. (1996). New evidence for an inflammatory component in diarrhea caused by selected new, live attenuated cholera vaccines and by El Tor and Q139 *Vibrio cholerae*. *Infection and Immunity*, *64*(6), 2362–2364.

Simsek-ege, F. A., Bond, G. M., & Stringer, J. (2003). Polyelectrolye Complex Formation Between Alginate and Chitosan as a Function of pH. *Journal of Applied Polymer Science*, *88*(2), 346–351

Singh, M., & O'Hagan, D. (1999). Advances in vaccine adjuvants. *Nature Biotechnology*, *17*, 1075–1081.

Sone, T., Nagamori, E., Ikeuchi, T., & Mizukami, A. (2002). A Novel Gene Delivery System in Plants with Calcium Alginate Micro-Beads. *Journal of Bioscience and Bioengineering*, *94*(1), 87–91.

Sosnik, A. (2014). Alginate Particles as Platform for Drug Delivery by the Oral Route: State-of-the-Art. *ISRN Pharmaceutics*, *2014*, 1–17.

Spagnuolo, A. M., DiRita, V., & Kirschner, D. (2012). A model for *Vibrio cholerae* colonization of the human intestine. *Journal of Theoretical Biology*, *289*, 247–258. https://doi.org/10.1016/j.jtbi.2011.08.028.A

Spira, W. M., & Sack, R. B. (1982). Kinetics of Early Cholera Infection in the Removable Intestinal Tie-Adult Rabbit Diarrhea Model. *Infection and Immunity*, *35*(3), 952–957.

Spira, W. M., Sack, R. B., & Froehlich, J. L. (1981). Simple Adult Rabbit Model for *Vibrio cholerae* and Enterotoxigenic *Escherichia coli* Diarrhea. *Infection and Immunity*, *32*(2), 739–747.

Stockwell, A. F., & Davis, S. S. (1986). *In vitro* evaluation of alginate gel systems as sustained release drug delivery systems. *Journal of Controlled Release*, *3*(1–4), 167–175.

Stokes, N. R., Zhou, X., Meltzer, S. J., & Kaper, J. B. (2004). Transcriptional Responses of Intestinal Epithelial Cells to Infection with *Vibrio cholerae*. *Infection and Immunity*, *72*(7), 4240–4248. https://doi.org/10.1128/IAI.72.7.4240

Storni, T., Kundig, T. M., Senti, G., & Johansen, P. (2005). Immunity in response to particulate antigen-delivery systems. *Advanced Drug Delivery Reviews*, *57*(3), 333–355. https://doi.org/10.1016/j.addr.2004.09.008

Stuart, L. M., & Ezekowitz, R. A. B. (2005). Phagocytosis: Elegant Complexity Review. *Immunity*, *22*(5), 539–550. https://doi.org/10.1016/j.immuni.2005.05.002

Swearengen, J. R. (2018). Choosing the right animal model for infectious disease research. *Animal Models and Experimental Medicine*, *1*(2), 100–108. https://doi.org/10.1002/ame2.12020

Syahril, N. A., Mariana, N. S., Sidhu, H. S., & Rosli, R. (2002). *In Vitro* Expression of the *ctxB* Toxin Gene Towards the Development of a DNA Vaccine Against Cholera. *Molecular Biology Today*, *3*, 71–77.

Sone, T., Nagamori, E., Ikeuchi, T., Mizukami, A., Takakura, Y., Kajiyama, S., Fukusaki, E., Harashima, S., Kobayashi, A. & Fukui, K. (2002). A Novel Gene Delivery System in Plants with Calcium Alginate Micro-Beads. *Journal of Bioscience and Bioengineering*, *94*(1), 87–91.

Tanaka, A., & Nakajima, H. (1990). Application of Immobilized Growing Cells. *Advances in Biochemical Engineering/Biotechnology*, *42*, 97–131.

Tønnesen, H. H., & Karlsen, J. (2002). Alginate in Drug Delivery Systems Alginate in Drug Delivery Systems. *Drug Development and Industrial Pharmacy*, *28*(6), 621–630. https://doi.org/10.1081/DDC-120003853

Tian, J., Sun, X., & Chen, X. (2008). Formation and oral administration of alginate microspheres loaded with pDNA coding for lymphocystis disease virus (LCDV) to Japanese flounder. *Fish & Shellfish Immunology*, *24*(5), 592–599. https://doi.org/10.1016/j.fsi.2008.01.009

Trach, D. D., Clemens, J. D., Ke, N. T., Thuy, H. T., Son, N. D., Canh, D. G., Hang, P. V. D. & Rao, M. R. (1997). Field trial of a locally produced, killed, oral cholera vaccine in Vietnam. *The Lancet*, *349*, 231–235.

Tsai, B., & Rapoport, T. A. (2002). Unfolded cholera toxin is transferred to the ER membrane and released from protein disulfide isomerase upon oxidation by Ero1. *The Journal of Cell Biology*, *159*(2), 207–215. https://doi.org/10.1083/jcb.200207120

Tsukada, Y., Hara, K., Bando, Y., Huang, C. C., Kousaka, Y., Kawashima, Y., Morishita, R. & Tsujimoto, H. (2009). Particle size control of poly(DL-lactide-co-glycolide) nanospheres for sterile applications. *International Journal of Pharmaceutics*, *370*, 196–201. https://doi.org/10.1016/j.ijpharm.2008.11.019

van Loon, F. P. L., Clemens, J. D., Chakraborty, J., Rao, M. R., Kay, B. A., Sack, D. A., Yunus, M., Ali, M., Svennerholm, A-M. & Holmgren, J. (1996). Field trial of inactivated oral cholera vaccines in Bangladesh: results from 5 years of follow-up. *Vaccine*, *14*(2), 162–166.

Vogel, R., Pal, A. K., Jambhrunkar, S., Patel, P., Thakur, S. S., Reátegui, E., Parekh, H. S., Saá, P., Stassinopoulos, A. & Broom, M. F. (2017). High-Resolution Single Particle Zeta Potential Characterisation of Biological Nanoparticles using Tunable Resistive Pulse Sensing. *Scientific Reports*, *7*(17479), 1–13. https://doi.org/10.1038/s41598-017-14981-x

Wack, A., & Rappuoli, R. (2005). Vaccinology at the beginning of the 21st century. *Current Opinion in Immunologyogy*, *17*(4), 411–418. https://doi.org/10.1016/j.coi.2005.05.005

Wang, B., Ugen, K. E., Srikantan, V., Agadjanyan, M. G., Dang, K., Refaeli, Y., Sato, A. I., Boyer, J., Williams, W. V. & Weiner, D. B. (1993). Gene inoculation generates immune responses against human immunodeficiency virus type 1. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(9), 4156–4160.

Wang, C., Ge, Q., Ting, D., Nguyen, D., Shen, H., Chen, J., Eisen, H. N., Heller, J., Langer, R. & Putnam, D. (2004). Molecularly engineered poly(ortho ester) microspheres for enhanced delivery of DNA Vaccine.

Nature Materials, *3*(3), 190–196. https://doi.org/10.1038/nmat1075

Wang, S., & Lu, S. (2013). DNA Immunization. *Current Protocols in Microbiology*, *31*, 1–28. https://doi.org/10.1002/9780471729259.mc1803s31.DNA

Weil, A. A., Arifuzzaman, M., Bhuiyan, T. R., LaRocque, R. C., Harris, A. M., Kendall, E. A., Hossain, A., Tarique, A. A., Sheikh, A., Chowdury, F., Khan, A. I., Murshed, F., Parker, K. C., Banerjee, K. K., Ryan, E. T., Harris, J. B., Qadri, F. & Calderwood, S. B. (2009). Memory T-Cell Responses to *Vibrio cholerae* O1 Infection. *Infection and Immunity*, *77*(11), 5090–5096. https://doi.org/10.1128/IAI.00793-09

Weil, A. A., Begum, Y., Chowdhury, F., Khan, A. I., Leung, D. T., LaRocque, R. C., Charles, R. C., Ryan, E. T., Calderwood, S. B., Qadri, F. & Harris, J. B. (2014). Bacterial shedding in household contacts of cholera patients in Dhaka, Bangladesh. *American Journal of Tropical Medicine and Hygiene*, *91*(4), 738–742. https://doi.org/10.4269/ajtmh.14-0095

Wischke, C., & Schwendeman, S. P. (2008). Principles of encapsulating hydrophobic drugs in PLA/PLGA microparticles. *International Journal of Pharmaceutics*, *364*(2), 298–327. https://doi.org/10.1016/j.ijpharm.2008.04.042

World Health Organization. (2014). *Knowledge, Attitudes, and Practices (KAP) Surveys during Cholera Vaccination Campaigns: Guidance for Oral Cholera Vaccine Stockpile Campaigns*.

World Health Organization. (2017a). *Deployments from the oral cholera vaccine stockpile, 2013-2017* (Vol. 13).

World Health Organization. (2017b). *Weekly epidemiological record*.

Xiang, S. D., Scholzen, A., Minigo, G., David, C., Apostolopoulos, V., Mottram, P. L., & Plebanski, M. (2006). Pathogen recognition and development of particulate vaccines: Does size matter? *Methods*, *40*(1), 1–9. https://doi.org/10.1016/j.ymeth.2006.05.016

Yeh, P., Ellens, H., & Smith, P. L. (1998). Physiological considerations in the design of particulate dosage forms for oral vaccine delivery. *Advanced Drug Delivery Reviews*, *34*(2–3), 123–133.

Zhang, H., Tumarkin, E., Peerani, R., Nie, Z., Sullan, R. M. A., Walker, G. C., & Kumacheva, E. (2006). Microfluidic Production of Biopolymer Microcapsules with Controlled Morphology. *Journal of the American Chemical Society*, *128*(37), 12205–12210.