Pentavalent outer membrane vesicles of *V. cholerae* induce adaptive immune response and protective efficacy in both adult and passive suckling mice model

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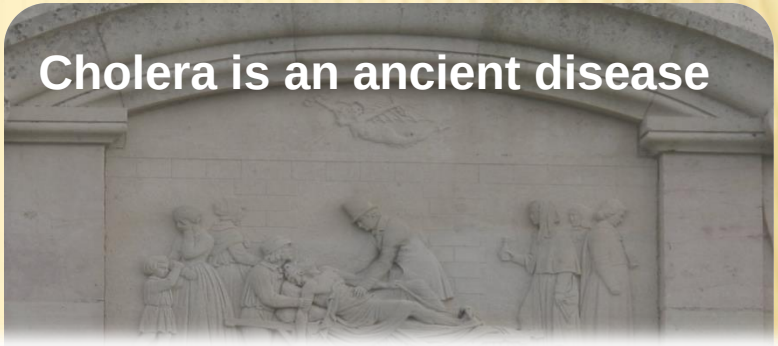
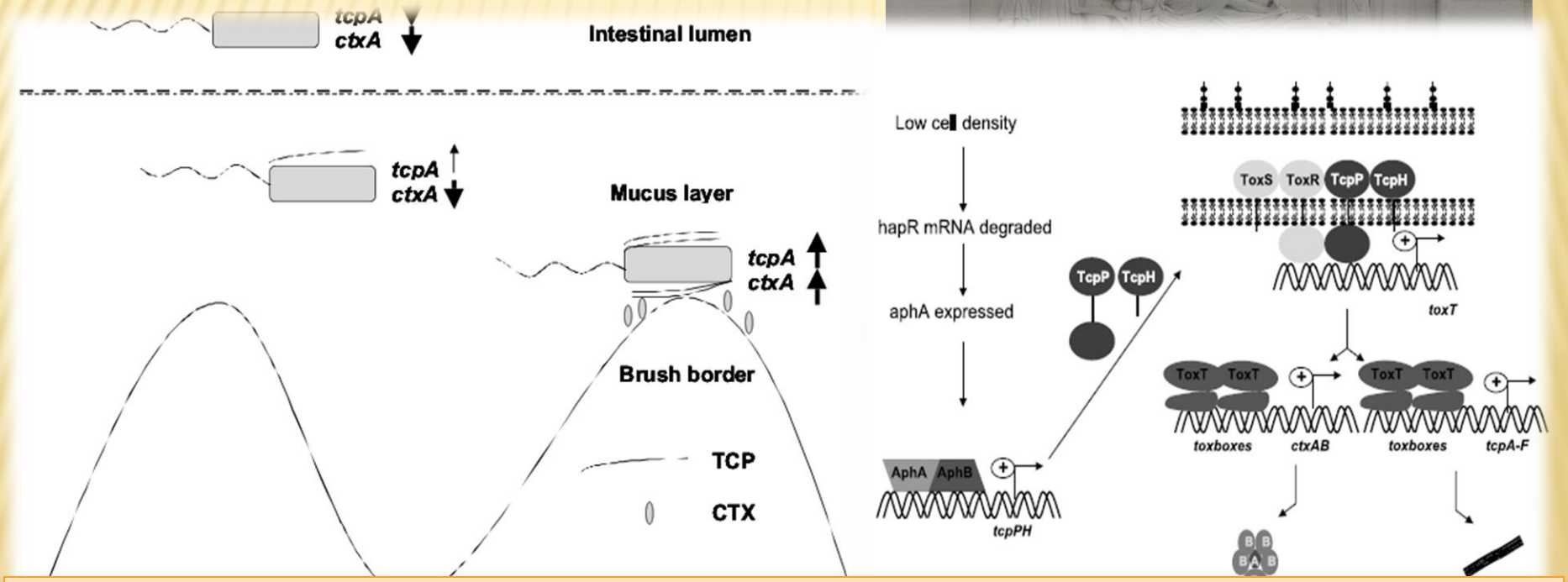
What is Cholera ?

Since 1817, cholera is one of the major clinical problems in many of the developing countries in Asia, Africa and South America

In 2013, Haiti cholera outbreaks occurs causes death 8,231 Haitians



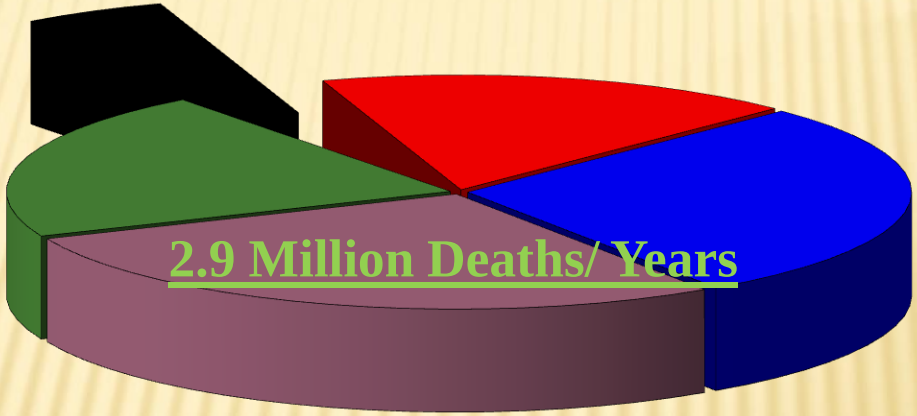


Soon after oral ingestion, *V. cholerae* reaches to the small bowel, its primary site of colonization, and induces virulence factors - toxin-coregulated pilus (TCP) and cholera toxin (CT).

ENTERIC PATHOGENS OF GLOBAL IMPORTANCE

Cholera
(150,000 deaths)

ETEC diarrhea
(500,000 deaths)



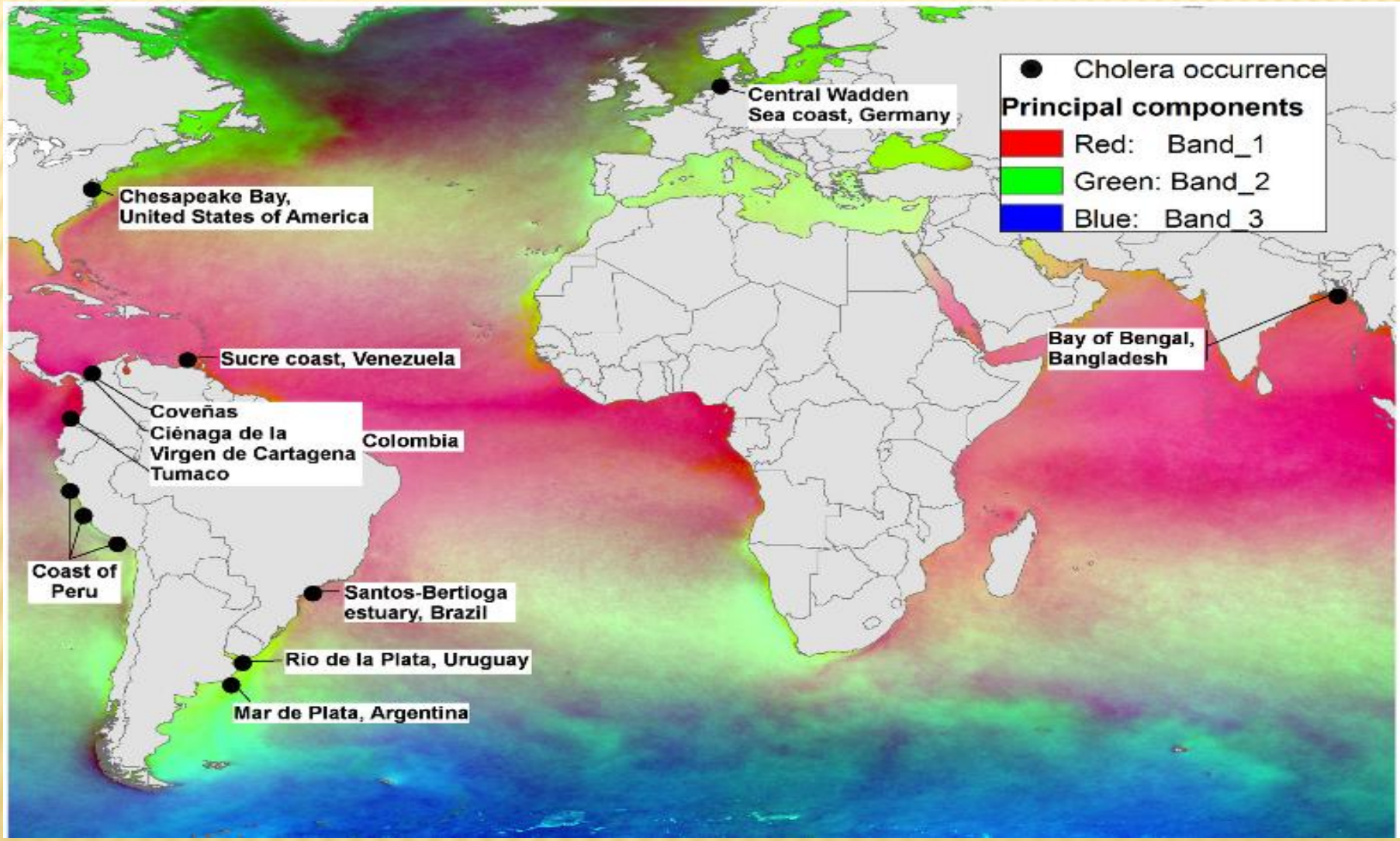
Typhoid fever
(600,000 deaths)

Shigellosis
(800,000 deaths)

Rotavirus diarrhea
(800,000 deaths)

*World Health Organization GBD_report_2014update_part4

Global occurance of *V.cholerae* infection



L.E. Escobar et al. / Acta Tropica 149 (2015) 202–211

Typical Sign of Cholera

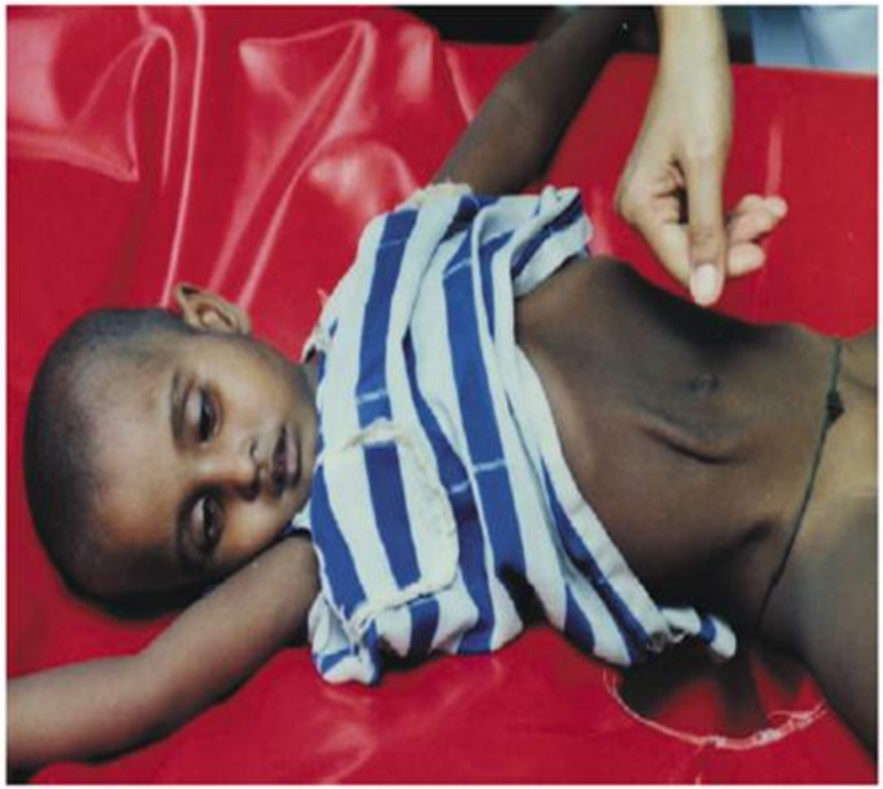


Figure : **A child, lying on a cholera cot, showing typical signs of severe dehydration from cholera**
The patient has sunken eyes, lethargic appearance, and poor skin turgor, but within 2 h was sitting up, alert, and eating normally.

Management of patients with suspected cholera

Assess for dehydration.

Rapidly rehydrate the patient with intravenous Ringer's solution for severely dehydrated patients or ORS for those with less severe dehydration; use rice-based ORS if possible.

Severely dehydrated patients require replacement of 10% of their bodyweight within 2–4 h.

Use cholera cot (if possible) to monitor stool output; monitor status of hydration and monitor severity of purging frequently.

Maintain hydration by replacing continuing fluid losses until diarrhoea stops.

Give an oral antibiotic (eg, doxycycline) to dehydrated patients as soon as vomiting stops.

Provide food as soon as patient is able to eat (within a few hours).

Sack, David, *et al*. 2004. Seminar: Cholera. The Lancet. 363: 223-233.

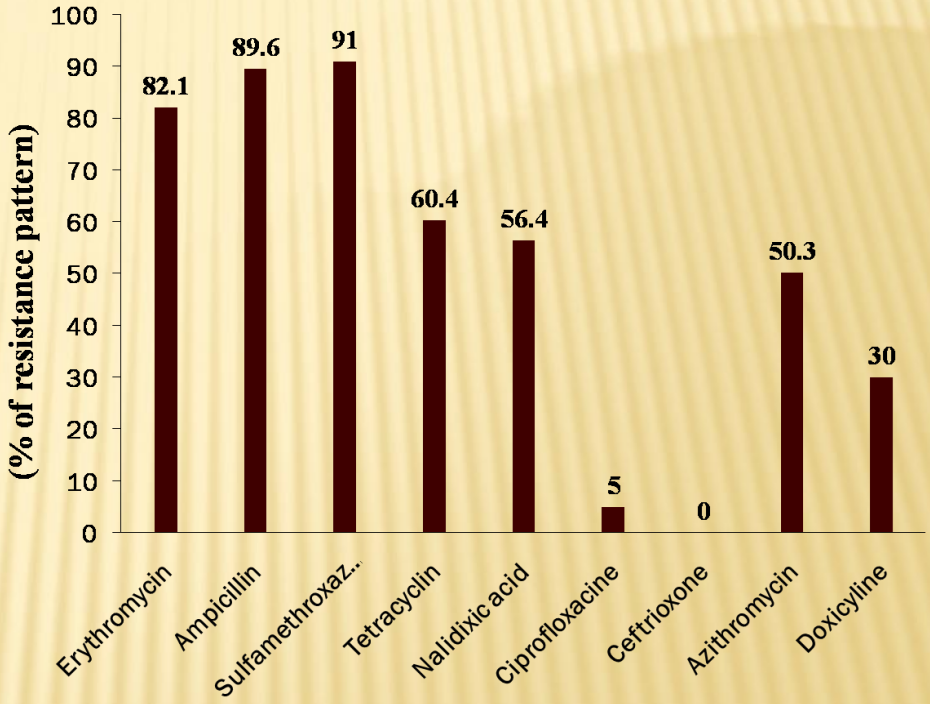
Development of multi drug resistance *V. cholerae in Asia*

Drug of Choice

Guidelines for Cholera Treatment with Antibiotics				
Organization	**Recommendation**	**First-line drug choice**	**Alternate drug choices**	**Drug choices for special populations**
World Health Organization [21]	Antibiotic treatment for cholera patients with severe dehydration only	Doxycycline	Tetracycline	Erythromycin is recommended drug for children
Pan American Health Organization [22]	Antibiotic treatment for cholera patients with moderate or severe dehydration	Doxycycline	Ciprofloxacin Azithromycin	Erythromycin or azithromycin recommended as first-line drugs for pregnant women and children Ciprofloxacin and doxycycline recommended as second-line drugs for children
International Centre for Diarrhoeal Disease Research, Bangladesh [23]	Antibiotic treatment for cholera patients with some or severe dehydration	Doxycycline	Ciprofloxacin Azithromycin Cotrimoxazole	Erythromycin recommended as first-line drug for children and pregnant women
Medicins Sans Frontieres [24]	Antibiotic treatment for severely dehydrated patients only	Doxycycline	Erythromycin Cotrimoxazole Chloramphenicol Furazolidone	

According to the CDC

Drug resistance pattern



Yu L,et al.. PLoS One. 2014;7(6):, Faruque AS,et al. J Health Popul Nutr. 2007 Jun;25(2):241-3., . Tariq Bhat et al. JK-Practitioner ,2012

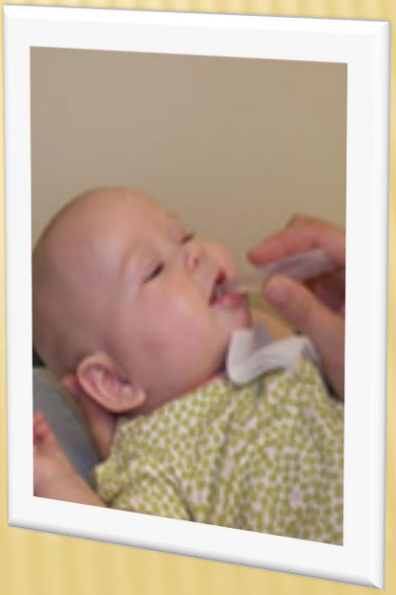
Vaccination will be the ultimate solution

WHAT IS VACCINE

➢***Attenuated*** **or** ***killed*** **microorganisms or proteins derived from them, administered for the prevention, treatment, or amelioration of infectious diseases**

THE IDEAL VACCINE

- **Immunogenic**
- **Long lasting immunity**
- **Safe**
- **Stable in field conditions**
- **Combined**
- **Single dose**
- **Affordable (and accessible) to all**



Oral Cholera Vaccine

WHO approved two licensed vaccine

Drawback:

Recent epidemic in Haiti and Zimbabwe was due to El Tor biotype.

Both Durakol and Shancol vaccine have questionable composition using three preparation of classical O1 strain and only one preparation of El Tor strain

Short duration of Protection.

Although Shancol contains large propotion of killed *V. cholerae* O139 cells, field trial showed the protective efficacy was low in both adult and children under 18 (less than 30%)

Nee

Need:
Scientists, academicians and public health experts have been searching for a suitable candidate vaccine to combat cholera

Outer membrane vesicles of gram negative bacteria as a next generation vaccine candidate

Infection and Immunity

Comparison and Correlation of *Neisseria meningitidis* Serogroup B Immunologic Assay Results and Human Antibody Responses following Three Doses of the Norwegian Meningococcal Outer Membrane Vesicle Vaccine MenBvac

Jamie Findlow, Stephen Taylor, Audun Aase, Rachel Horton, Robert Heyderman, Jo Southern, Nick Andrews, Rita Borchha, Ewan Harrison, Ann Lowe, Emma Boxer, Charlotte Heaton, Paul Balmer, Ed Kaczmarski, Philipp Oster, Andrew Gorringe, Ray Borrow and Elizabeth Miller
Infect. Immun. 2006, 74(8):4557. DOI: 10.1128/IAI.00466-06.

Infection and Immunity

Naturally Produced Outer Membrane Vesicles from *Pseudomonas aeruginosa* Elicit a Potent Innate Immune Response via Combined Sensing of Both Lipopolysaccharide and Protein Components

Terri N. Ellis, Sara A. Leiman and Meta J. Kuehn
Infect. Immun. 2010, 78(9):3822. DOI: 10.1128/IAI.00433-10.
Published Ahead of Print 6 July 2010.

OPEN ACCESS Freely available online

PLoS one

Outer Membrane Vesicles as a Candidate Vaccine against Edwardsiellosis

Seong Bin Park, Ho Bin Jang, Seong Won Nho, In Seok Cha, Jun-ichi Hikima, Maki Ohtani, Takashi Aoki*, Tae Sung Jung*

Aquatic Biotechnology Center, College of Veterinary Medicine, Gyeongsang National University, Jinju, South Korea

Vaccine 29 (2011) 1649–1656

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

ELSEVIER

Outer membrane vesicles obtained from *Bordetella pertussis* Tohama expressing the lipid A deacylase PagL as a novel acellular vaccine candidate

Cristian J.A. Asensio[a,1], María Emilia Gaillard[a,1], Griselda Moreno[c], Daniela Bottero[a], Eugenia Zurita[a], Martin Rumbo[c], Peter van der Ley[b], Arno van der Ark[b], Daniela Hozbor[a,*]

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Immunogenicity and protective efficacy of *Vibrio cholerae* outer membrane vesicles in rabbit model

FREE

Nivedita Roy[1], Soumik Barman[1], Amit Ghosh[2], Amit Pal[2], Krishnendu Chakraborty[3], Santa Sabuj Das[3], Dhira Rani Saha[4], Shinji Yamasaki[5], Hemanta Koley[1]

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Issue

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1556-6811/11/$12.00 doi:10.1128/CVI.05217-11

Outer Membrane Vesicles Induce Immune Responses to Virulence Proteins and Protect against Colonization by Enterotoxigenic *Escherichia coli*▽

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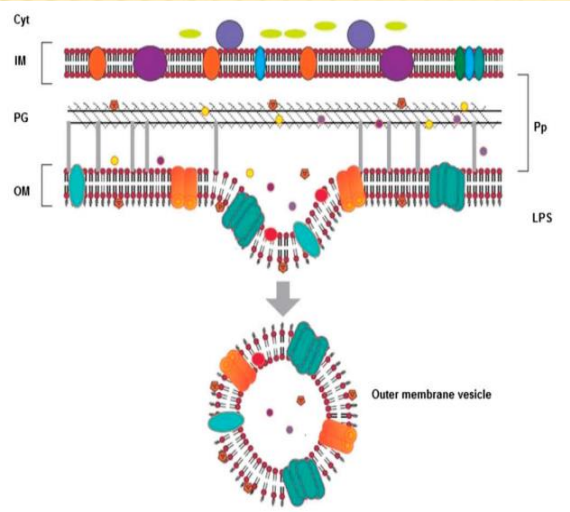
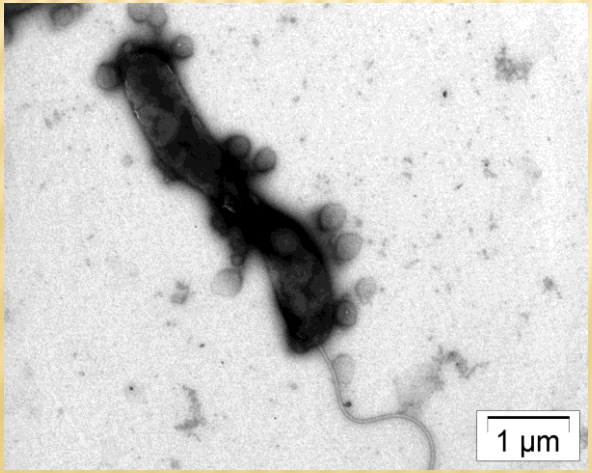
Departments of Medicine,[1] Comparative Medicine,[2] and Molecular Sciences,[3] University of Tennessee Health Sciences Center, Memphis, Tennessee; Department of Microbiology and Immunology, Miller School of Medicine, University of Miami, Miami, Florida[4]; and Veterans Affairs Medical Center, Memphis, Tennessee[5]

Received 15 June 2011/Returned for modification 26 July 2011/Accepted 1 September 2011

What is OMV?

Outer membrane vesicles (OMVs) produced by a wide variety of Gram-negative bacteria are spherical nanostructure (20-250 nm)





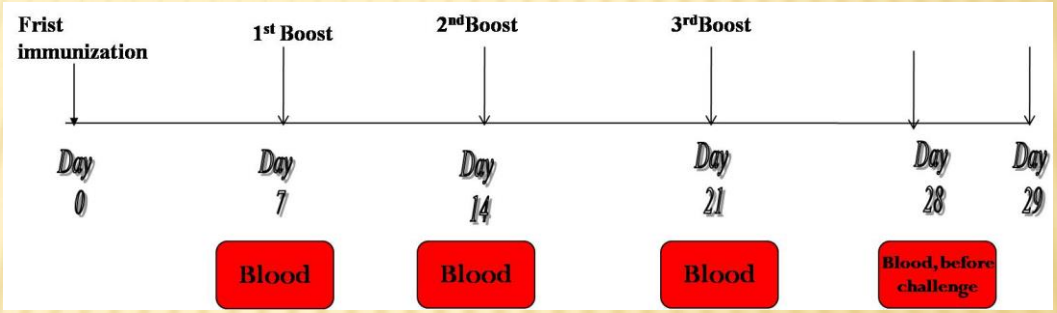
- **The surface of OMVs is thought to reflect the outer membrane composition of the bacterial cells**
- **OMVs are heterogeneous complexes of pathogen-associated molecular patterns (PAMPs), such as LPS, peptidoglycan, flagellin and CpG DNA, as well as other outer membrane proteins, virulence factors or immunomodulatory compounds that are important for pathogenesis**
- **Naturally secreted OMVs of *V.cholerae* contain both LPS and immunomodulatory proteins like OmpU, OmpT and OmpW.**

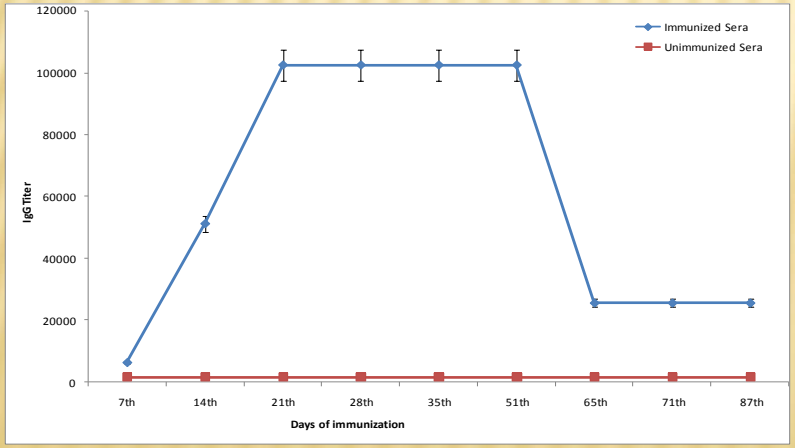
Back ground of my study

OMVs of *V.cholerae* O1 El Tor Inaba

- **Isolation Of OMVs from *V.cholerae O1* El Tor Inaba (N16961)**
- **To check its immunogenicity we immunized rabbit four times and check its protective efficacy by RITARD model.**

Immunization schedule

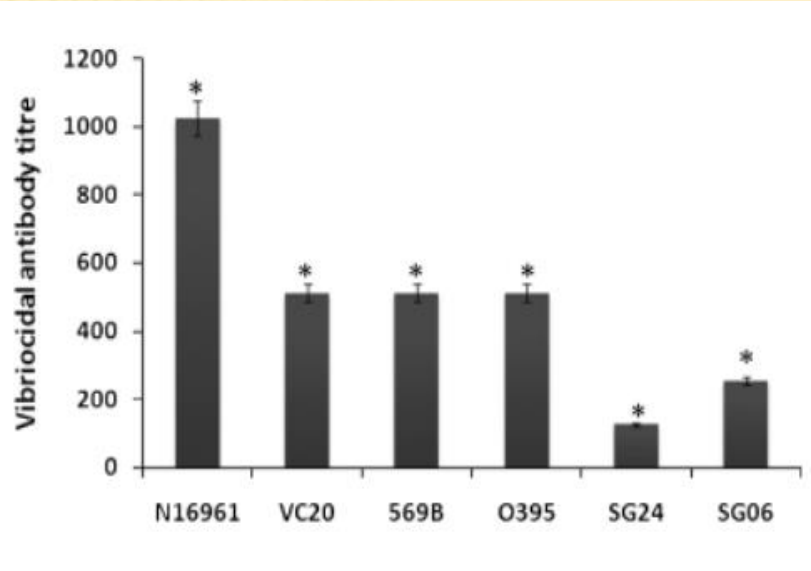




Serum IgG titer showed exponential increase during immunization and it picked on 21st day to 51st days significantly and persist at relatively high titer for 87th days.

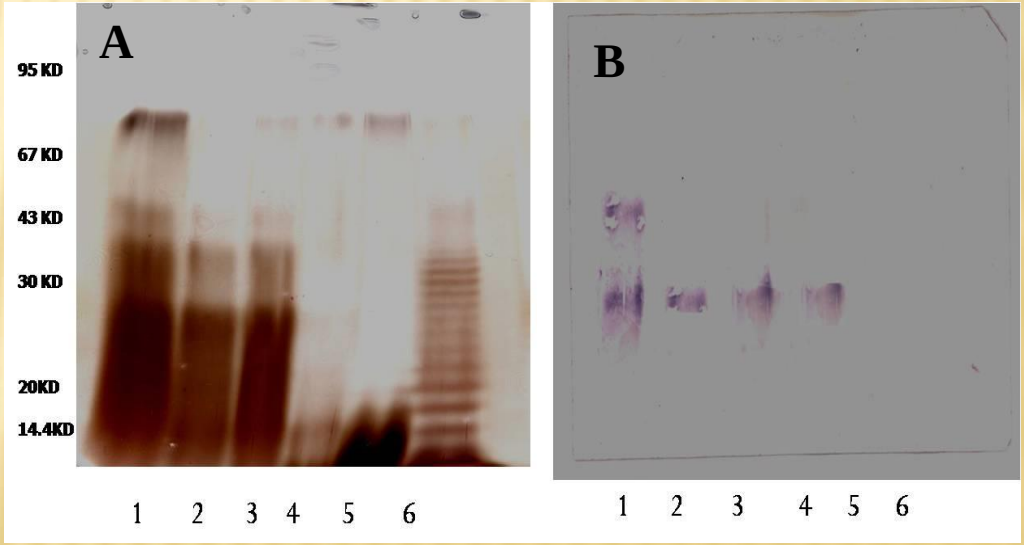
Koley H et al. 2010

Immunogenicity of OMVs from *V. cholerae* N16961



Vibriocidal activity of OMV-induced antibody. Rabbits were immunized with OMVs as described under Materials and methods.Antiserum was collected on day 28 and the homologous and fiveheterologousVibrio choleraestrains were used for the serum vibriocidal antibody assay as described above. Lane 1: N16961, lane 2: VC20, lane3: 569B, lane 4: O395, lane 5: SG24 and lane 6: SGO6

LPS separated by 10% SDS-PAGE (A) Western blot analysis (B) with rabbit antibody raised against N16961 OMV Lane1-N16961,Lane2-VC20,Lane3-569B,Lane4-O395,Lane5-SG24,Lane6-SGO6



Koley H et al. 2010

Protective efficacy study of OMVs from *V. cholerae* N16961 in rabbit

Homologous protection

Experimental Group	Immunogen	Challenged Strain	Number of animal used	Protective efficacy (%)
PBS Control	Nil	N16961	3	0% (0/3)
Immunized group of animal	OMVs of N16961	N16961	3	100% (3/3)

Heterologous protection

Challenge strain	Serogroup	Challenge dose	Experimental group	Experimental Rabbit	Protective efficacy (%)
VC 20	O1, El Tor Ogawa	$3\pm1\times10^9$/ml	Control	3	84.4
			Immunized	3	
569B	O1, Classical Inaba	$2\pm1\times10^9$/ml	Control	3	84.4
			Immunized	3	
SG 24	O139	$3\pm1\times10^9$/ml	Control	3	75.3
			Immunized	3	
SGO6	O6 (Non-O1,non O139)	$3\pm1\times10^9$/ml	Control	3	75.4
			Immunized	3	

Koley H et al. 2010

RESEARCH ARTICLE

Immunogenicity and protective efficacy of *Vibrio cholerae* outer membrane vesicles in rabbit model

Nivedita Roy[1], Soumik Barman[1], Amit Ghosh[2], Amit Pal[2], Krishnendu Chakraborty[3], Santa Sabuj Das[3], Dhira Rani Saha[4], Shinji Yamasaki[5] & Hemanta Koley[1]

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Correspondence: Hemanta Koley, Division Bacteriology, National Institute of Cholera and Enteric Diseases

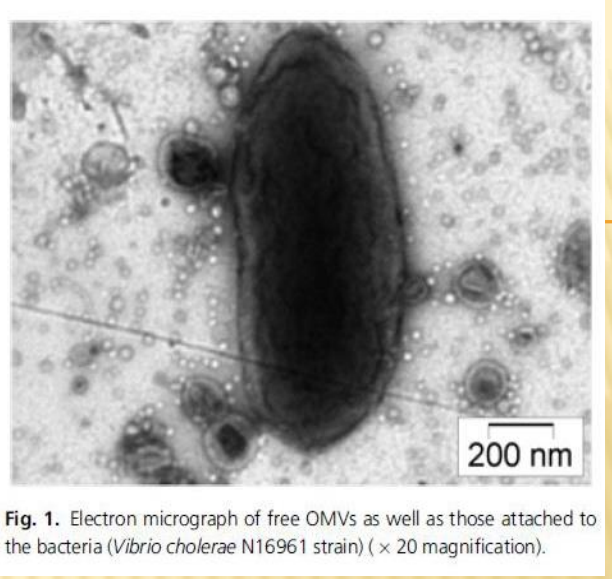


Fig. 1. Electron micrograph of free OMVs as well as those attached to the bacteria (*Vibrio cholerae* N16961 strain) (× 20 magnification).

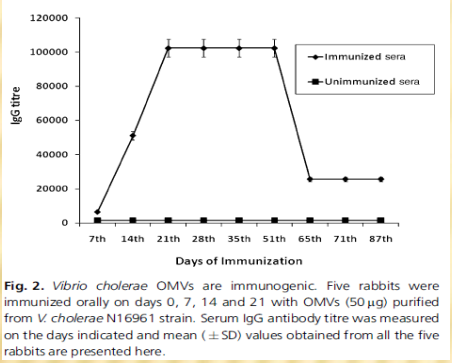
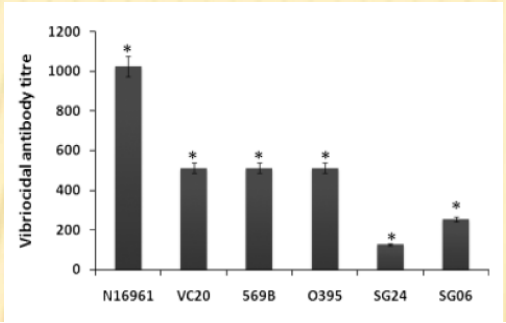
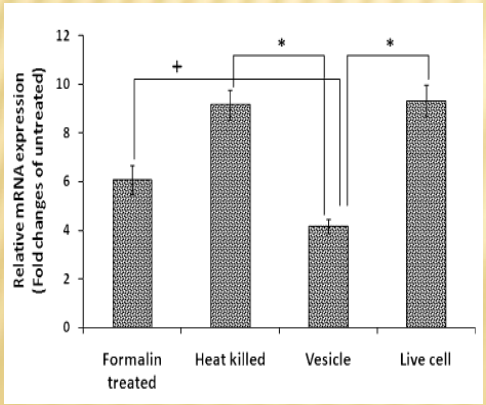
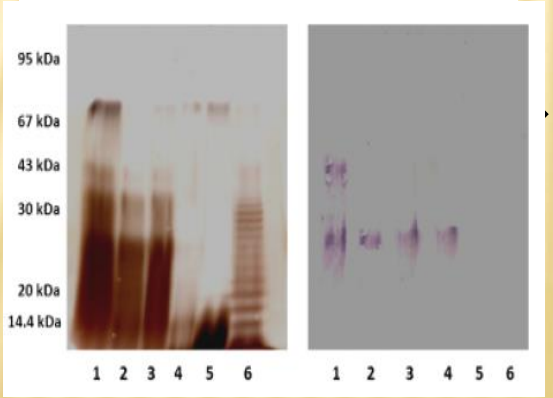


Fig. 2. *Vibrio cholerae* OMVs are immunogenic. Five rabbits were immunized orally on days 0, 7, 14 and 21 with OMVs (50 μg) purified from *V. cholerae* N16961 strain. Serum IgG antibody titre was measured on the days indicated and mean (±SD) values obtained from all the five rabbits are presented here.







- In the current study, we thoroughly investigated the safety, immunogenicity and protective efficacy of outer membrane vesicles (OMVs) derived from *V. cholerae* and their potential use as an orally administered candidate vaccine
- Oral immunization with purified (OMVs) of *Vibrio cholerae* conferred significant protective immunity against subsequent bacterial challenges
- Oral immunization with OMVs of *V. cholerae* may induce long-term immunity and may be useful as a ‘nonliving’ vaccine candidate for the future.

Though 100% homologous protection was quite satisfactory,

75% average heterologous protective efficacy bestowed by the OMVs of N16961 (O1 El Tor Inaba) was not at all promising from the purview of disease prevalence and severity.

Concept of Pentavalent Formulation

Protective immune response against *V. cholerae* are serogroup /serotype specific

Formulation of licensed heat-killed vaccine

Epidemic Scenario:

O1 El Tor Ogawa are predominant in w
O1 El Tor Inaba and O139 frequently o
continental.
O6 serogroup of Non O1,non O139 ser
predominant in India

Pentavalent formulation:

OMV of

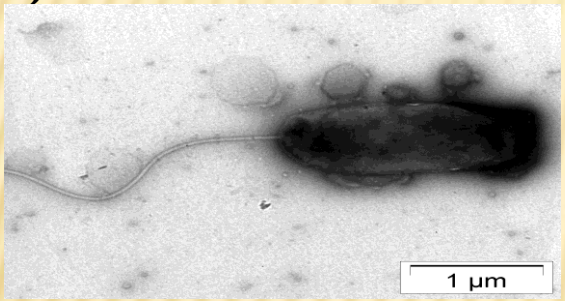
1) **O1 El Tor Inaba.**
2) **O1 El Tor Ogawa**
3) **O1 Classical Ogawa**
4) **O139**
5) **O6**

OMVs secretion from different wild type *V. cholerae* strains

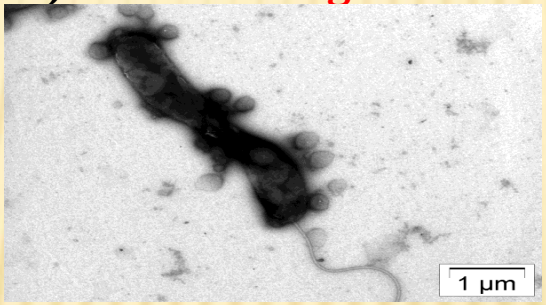
Bacterial strains used in this study.

Purpose	Serial no.	Strains name	Serogroup
CPMV preparation	1	O395	O1, Classical Ogawa
	2	N16961	O1, El Tor Inaba
	3	NLC8	O1, El Tor Ogawa variant
	4	K11575	O139
	5	SR O6	O6 (Non-O1,non-O139)

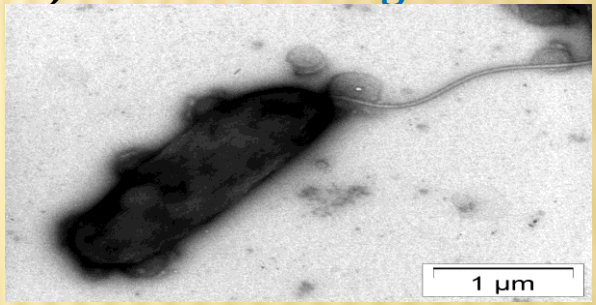
1) O1 El Tor Inaba



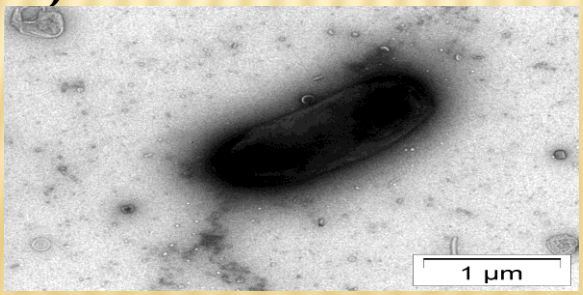
2) O1 El Tor Ogawa



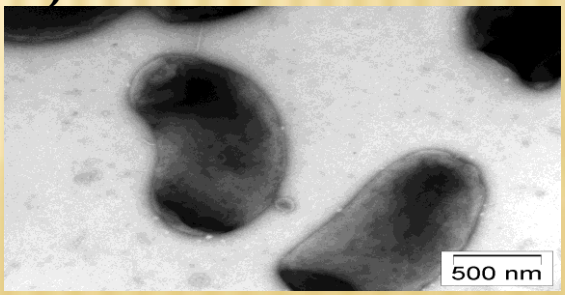
3) O1 Classical Ogawa



4) O139



5) O6



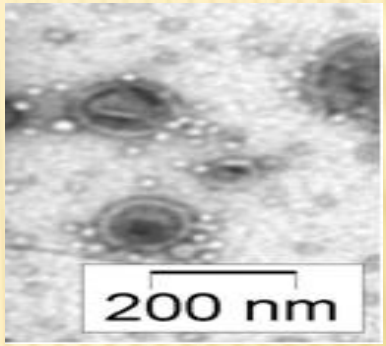
Preparation of outer membrane vesicles (OMVs)

8 to 10hr grown bacterial culture was centrifuged at 5000g for 30min.

Supernatant was passed through 0.45 and 0.22µm Millipore filter twice.

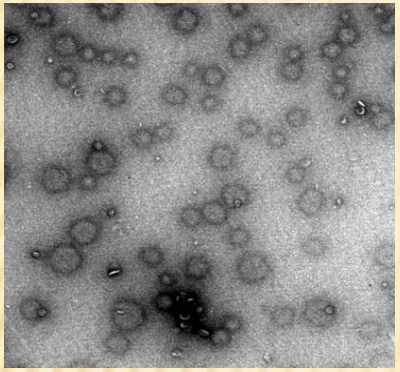
Resultant culture supernatant was subjected for ultra centrifugation (38,000 rpm for 3 hrs at 4° C)

Pellet was collected and resuspended in sterile PBS and the protein concentration was estimated by Bradford assay

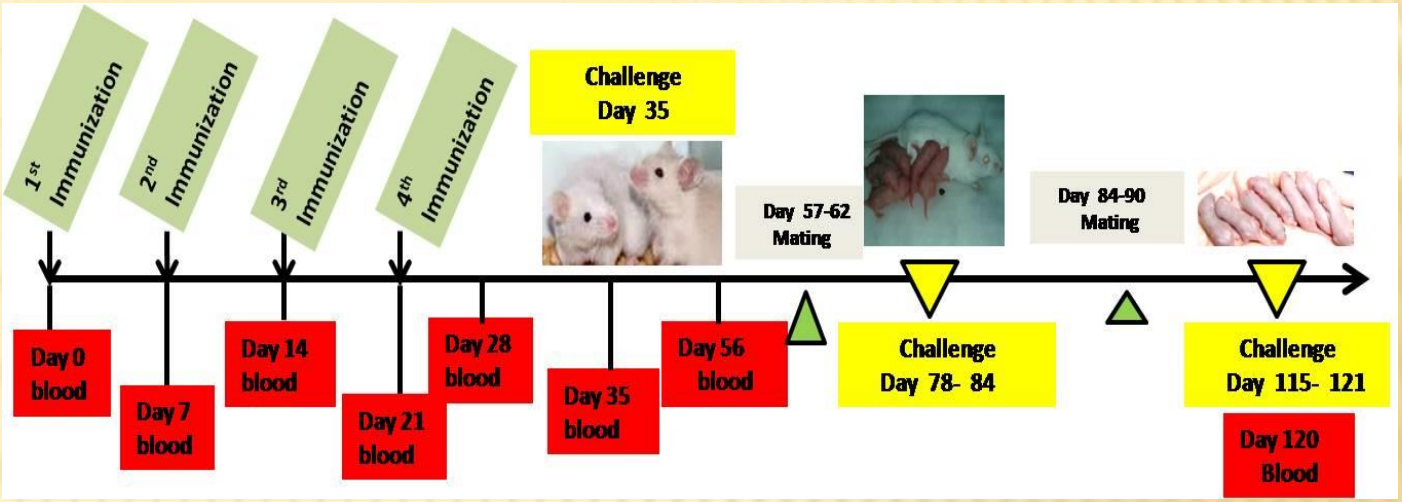


5 µl of OMV suspension was observed under Tecnai 12 (Bio Twin Transmission electron Microscope, FEI, Netherland) operating at 80 KV by negative staining method.

Study design



Purified CPMVs



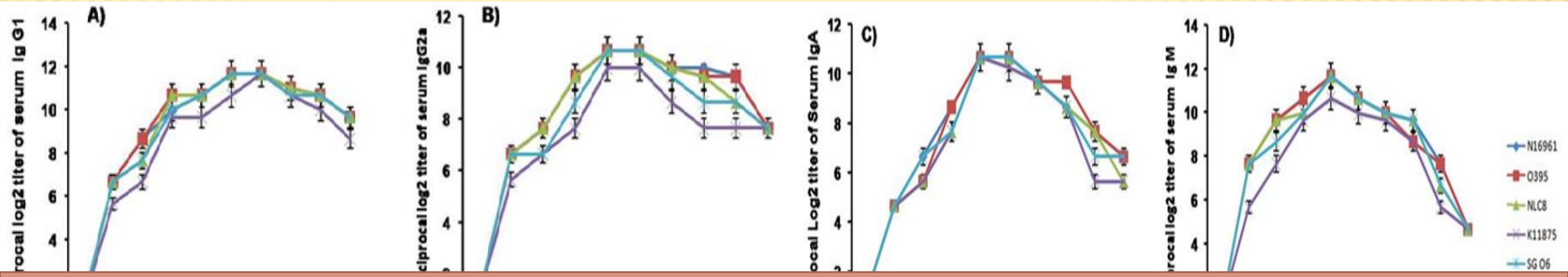
- Adult female mice were orally immunized with CPMVs(25µg/200µl).

- We examined immunogenicity of our newly formulated CPMVs.
  Adaptive immune response:
  i) Humoral Immunity
  ii) Cell mediated Immunity

- At day 35, protective efficacy were measured in adult female mice using mice illeal loop model.

- we also measured passive immunity in suckling mice model in different time interval

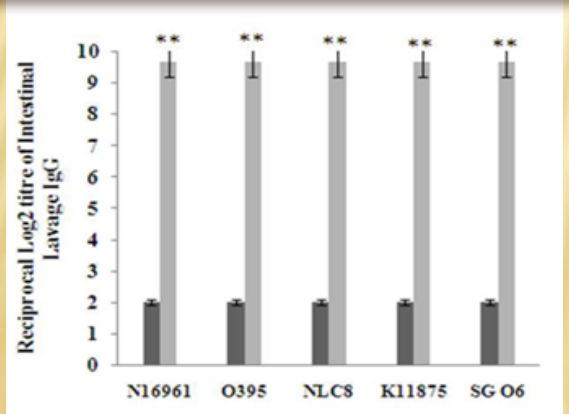
Induction of Humoral Immunity



Conclusion:

- **PentavalentOMVs of *V.cholerae* are potent immunogen significantly induce systemic and mucosal immune system**

- **The consistently high and sustained systematic and mucosal antibacterial immunoglobulins denote an underlying T helper response, which is essential for B cell activation, differentiation and generation of memory B cell response .**



between O1 and O139 serogroups was observed.

CPMVs immunization triggered higher IgG1 than IgG2a against each component of CPMVs which indicate Th2 cell mediated immune response.

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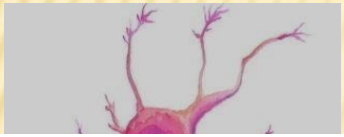
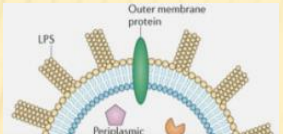
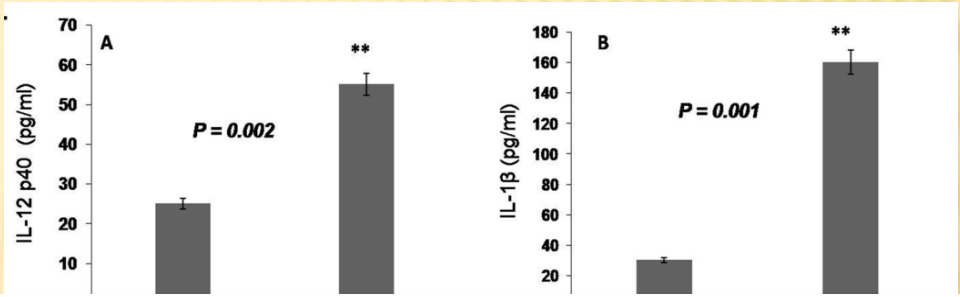
Activation of Dendritic cell

Secretion of T-cell polarizing cytokines from activated dendritic cells



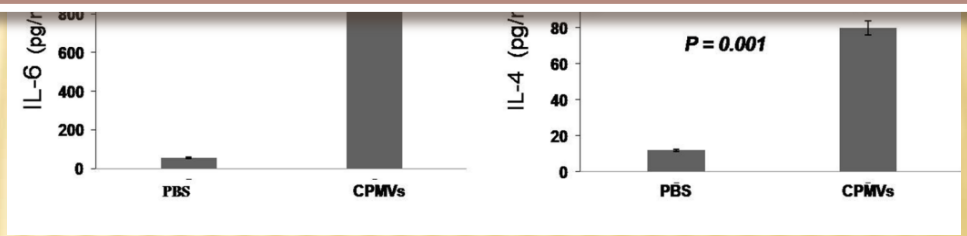




Conclusion:

Indication of generation Th2 and Th17 cell mediated immune response

BMDC **OMVs**



Th17 polarizing cytokines = IL-6,IL-12p40,IL-1ß
Th2 polarizing cytokines = IL-4

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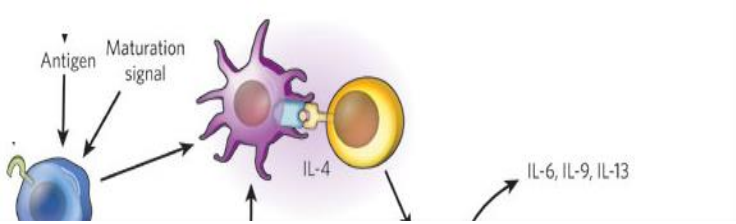
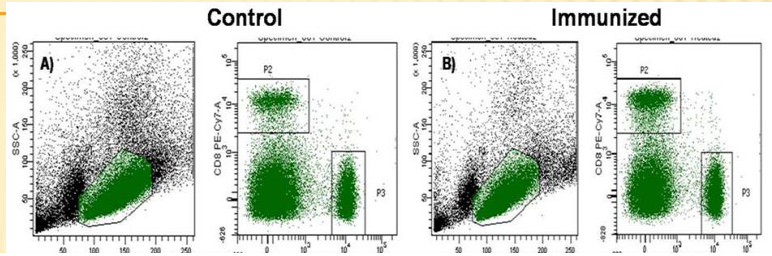
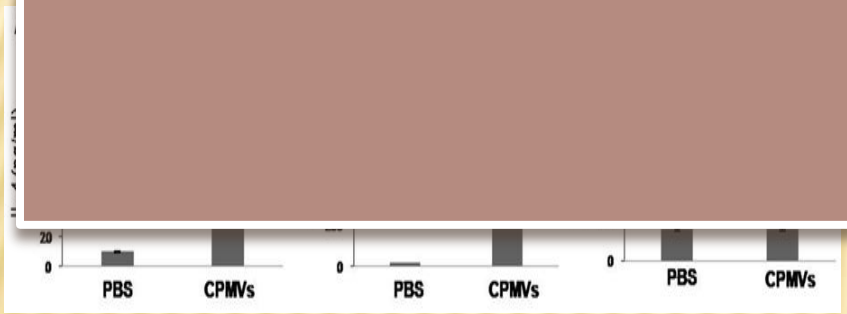
Induction of Cell Mediated Immune Response

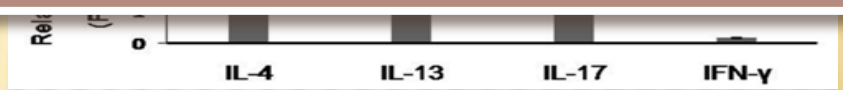




Conclusion:

Induction of long term Th2 and Th17 immunity could provide new therapeutic approaches for preventing *V. cholerae* induced inflammation and memory B cell response.





we also found secretion of IL-4, IL-13 (Th2) and IL-17 (Th17) from CD4+T cell of immunized mice.

Th2 cytokines such as IL-4, IL-5 are important stimulant for protective antibody production.

Newly identified Th cell lineage, Th17, which secrete IL-17, IL-21, IL-22, IL-6 and TNF-α, have protective role against intestinal bacterial infection .

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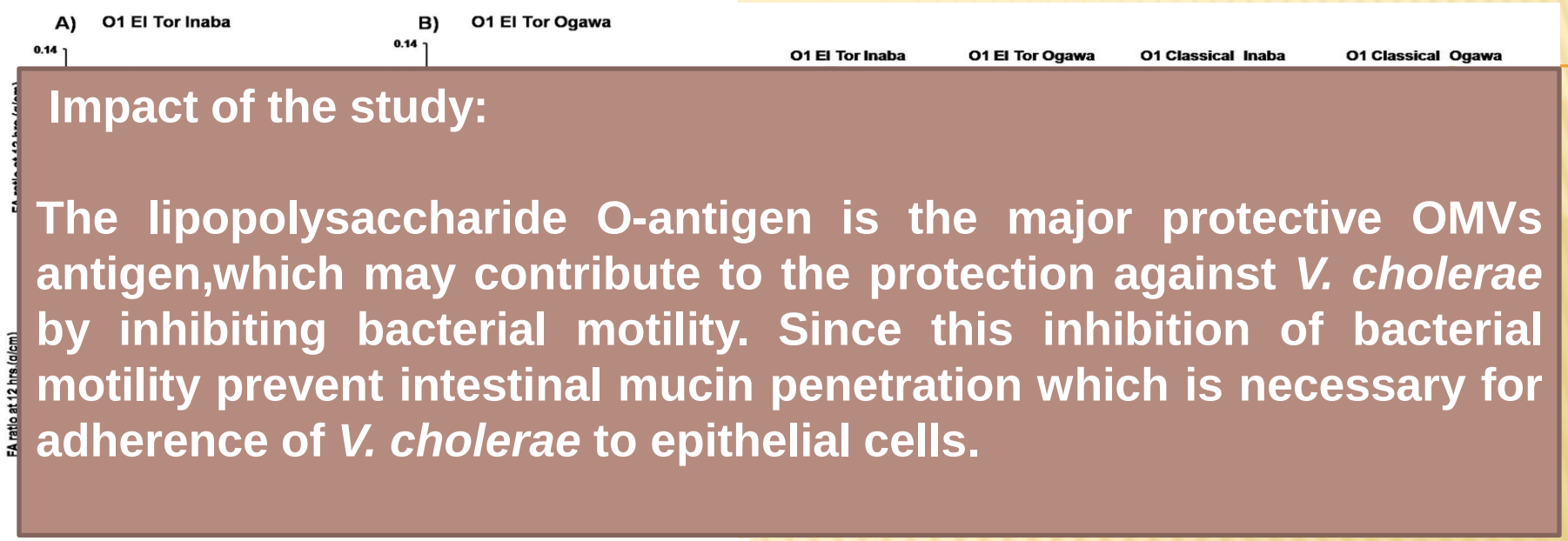
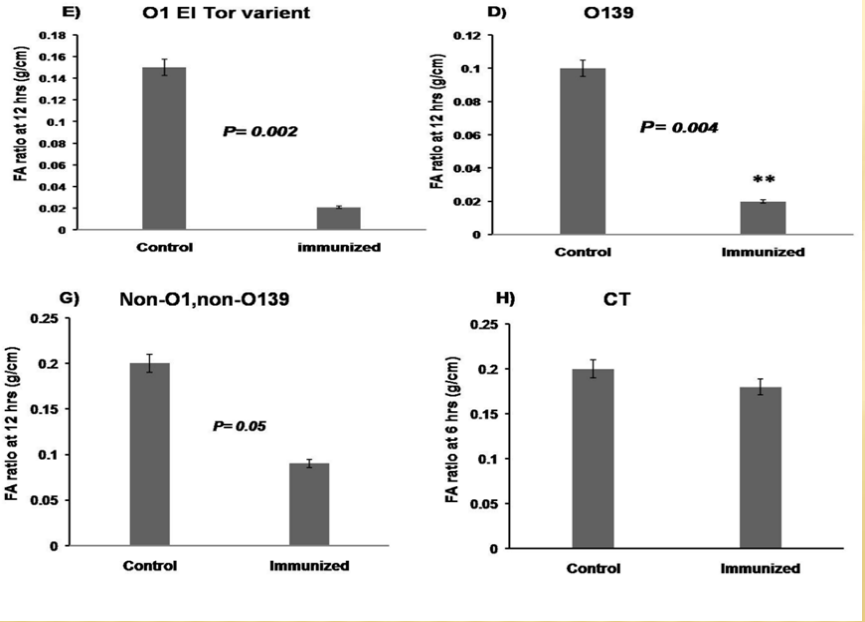
Protection Study in Adult Mice Model



Impact of the study:

The lipopolysaccharide O-antigen is the major protective OMVs antigen,which may contribute to the protection against *V. cholerae* by inhibiting bacterial motility. Since this inhibition of bacterial motility prevent intestinal mucin penetration which is necessary for adherence of *V. cholerae* to epithelial cells.



- **After four successive oral immunizations with CPMVs, protective efficacy was observed in adult mice ileal loop assaymeasuring fluid accumulation ratio of weight and length of ligated intestine significant**
- **We observed no amount of fluid accumulation in immunized group, whereas non-immunized group of animal's intestine showed significant amount of fluid accumulation.**
- **But fluid accumulation was observed in both loop of immunized and non-immunized mice introduced with cholera toxin**

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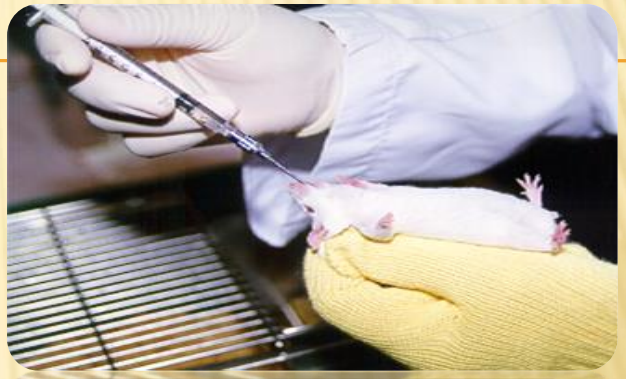
Protective Efficacy study- passive protection model

7 week old adult female mice

- Immunized with the CPMVs



Adult male mice

- Immunized female mice were mated with age matched males

Suckling mice

- Challenged with wild type virulent Wild type strains to study the protective efficacy of the multi-serotype OMVs vaccine formulation

Passive protection study

Protective efficacy study in neonatal mice.

Challenge strain	Serogroup	Challenge dose	Experimental group	Experimental neonatal	% of survival after first challenge	Protective efficacy (%)[a]	% of survival after second challenge	Protective efficacy (%)[a]
C6709	O1, El Tor Inaba	$2 \pm 1 \times 10^9$/ml	Control	10	10 (1/10)	100	10 (1/10)	100
			Immunized	20	100 (20/20)		100 (20/20)	
MAK757	O1, El Tor Ogawa	$3 \pm 1 \times 10^9$/ml	Control	10	10 (1/10)	94.4	10 (1/10)	94.4
			Immunized	20	95 (19/20)		95 (19/20)	
569B	O1, Classical Inaba	$2 \pm 1 \times 10^9$/ml	Control	10	20 (2/10)	94.4	20 (2/10)	87.5
			Immunized	20	95 (19/20)		85 (18/20)	
GP148	O1, Classical Ogawa	$5 \pm 1 \times 10^9$/ml	Control	10	10 (1/10)	100	10 (1/10)	100
			Immunized	20	100 (20/20)		85 (3/20)	
AM157	O1, El Tor Ogawa variant	$3 \pm 1 \times 10^9$/ml	Control	10	10 (1/10)	88.8	20 (2/10)	83.3
			Immunized	20	90 (18/20)		90 (17/20)	
SG 24	O139	$3 \pm 1 \times 10^9$/ml	Control	10	10 (1/10)	83.3	10 (1/20)	83.3
			Immunized	20	90 (18/20)		90 (18/20)	
SRK 16	O6 (Non-O1, non O139)	$3 \pm 1 \times 10^9$/ml	Control	10	10 (1/10)	94.4	10 (1/10)	87.5
			Immunized	20	85 (17/20)		85 (17/20)	

[a] Neonatal mice from the immunized and non-immunized dams were challenged by seven wild type strains of *V. cholerae* at different time periods. Values are means ± SD of three independent experiments. Protective efficacy was calculated as {[(percent deaths of non-immunized mice) – (percent deaths of immunized mice)]/[percent deaths of non-immunized mice]} × 100 [19].

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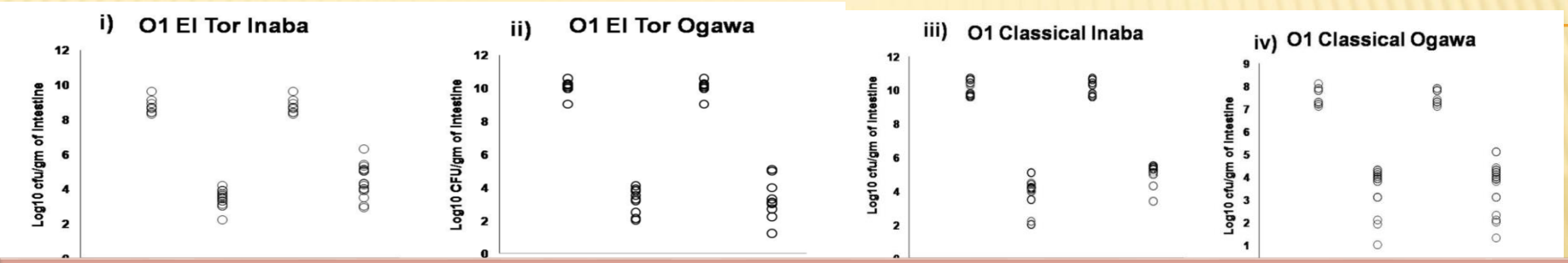
Passive protection study



From human perspective, effect of breast-feeding against cholera in endemic areas has been shown

The above result suggested that generation of long term passive protection would lead to increase significant protection in children against cholera during epidemics.

type strains. Passive protection study in suckling mice has supported our data by conferring higher rate of survivality and lesser intestinal colonization of *V. cholerae* when compared with neonates from non-immunized dams due totransferring anti-OMVs immunoglobulins through breast milk

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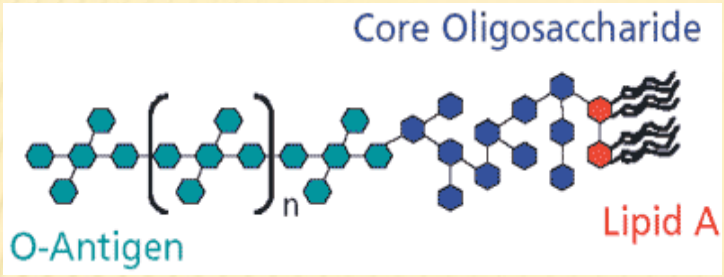
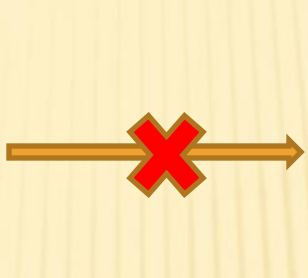
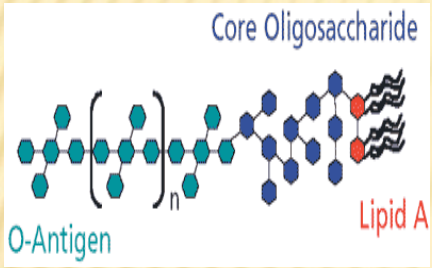
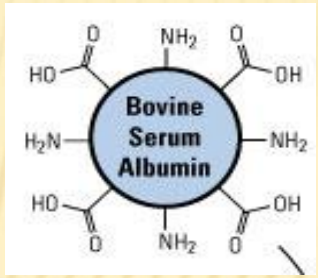
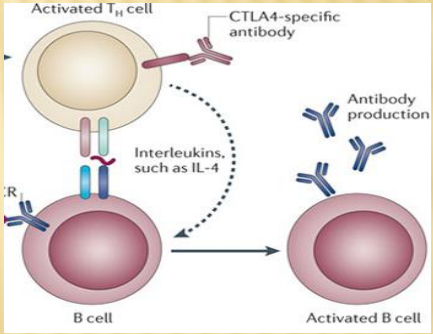
OMVs as a better immunogen

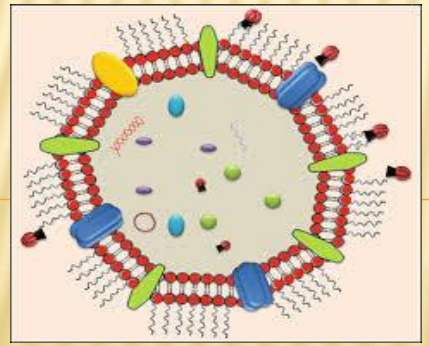




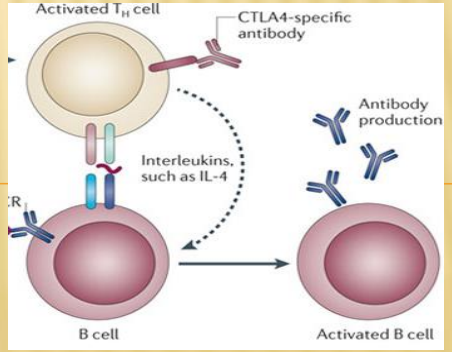








OMV



OMVs towards an ideal vaccine against *V. cholerae*

Immunogenic = 

Long lasting immunity 

Safe = 

Stable in field conditions = 

Combined= 

Dose = ??

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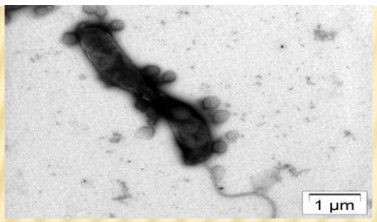
Microbes and Infection 17 (2015) 215–227

www.elsevier.com/locate/micinf

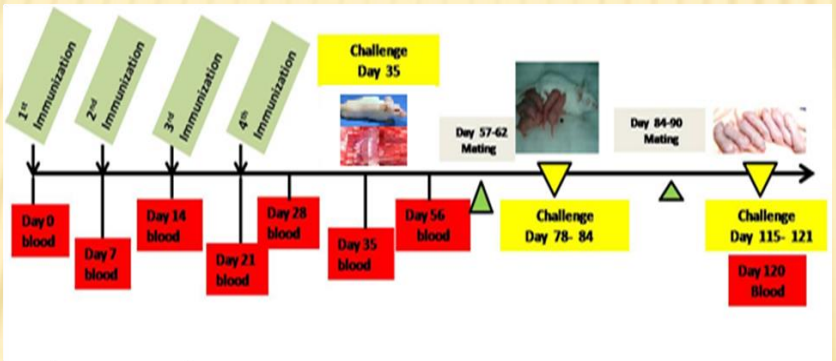
Original article

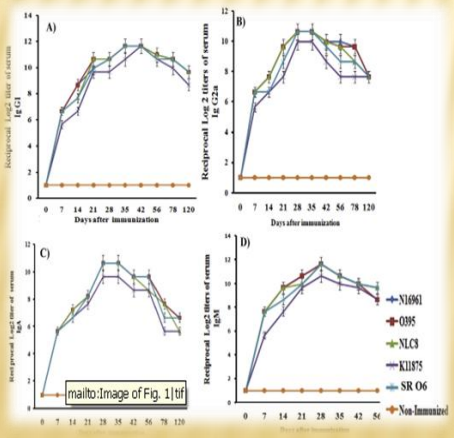
Pentavalent outer membrane vesicles of *Vibrio cholerae* induce adaptive immune response and protective efficacy in both adult and passive suckling mice models

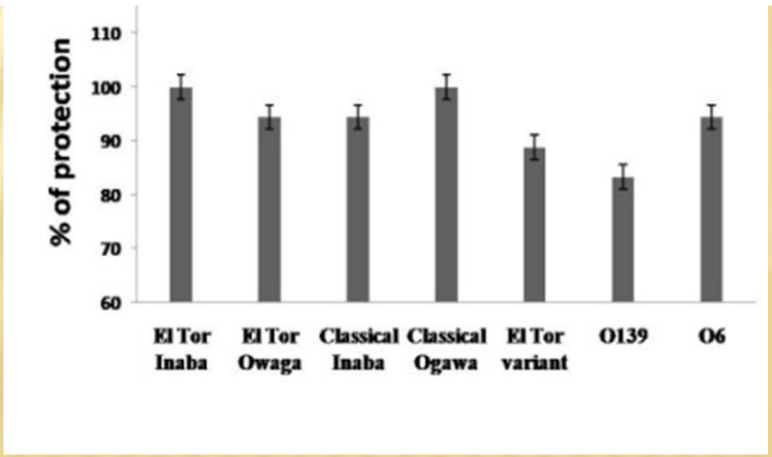
Ritam Sinha [a], Hemanta Koley [a,*], Dhrubajyoti Nag [a], Soma Mitra [a], Asish K. Mukhopadhyay [a],



Formulation of cholera pentavalent OMVs immunogen.
1) O1 El tor Inaba 2) O1 El tor Ogawa 3) O1 Classical Ogawa 4) Serogroup O139 5) Serogroup O6







Salient findings

- **Immunized sera showed significant increase different immunoglobulin during immunizationi.**
- **Induction of Th2/Th17 cell mediated adaptive immune response without any adjuvant, in adult mice.**
- **Up to 80-100% passive protection was observed in neonatal mice against diseases.**

Acknowledgement

My Lab Members

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THANK
YOU