Outer Membrane Vesicles: A Novel Particles for Next Generation Vaccine

Hemanta Koley, MSc PhD
Scientist
Division of Bacteriology,
National Institute of Cholera and Enteric Diseases
P-33 CIT Road, Scheme-XM, Beliaghata,
Kolkata, India

What is Shigellosis?

Shigellosis, diarrhoea with blood also known as bacillary dysentery or Marlow Syndrome, is a food borne illness caused by infection of bacteria, Shigella.

Shigellosis rarely occurs in animals other than humans, But physiological or surgical manipulation, it is possible to make shigellosis to the animal beings.

Shigella was discovered over 100 years ago by the Japanese microbiologist, Kiyoshi Shiga.







Dr. Kiyoshi Shiga: Discoverer of the Dysentery Bacillus



Born: February 7, 1871, Sendai, Miyagi Prefecture, Japan
Died: January 25, 1957, Tokyo, Japan
Education: University of Tokyo

Dr Kiyoshi Shiga was a Japanese physician and bacteriologist. He became famous for the discovery of *Shigella dysenteriae,* the bacillus causing dysentery, in 1897, during a severe epidemic in which more than 90,000 cases were reported, with a mortality rate approaching 30%. The bacterium Shigella was thus named after him, as well as the shiga toxin, which is produced by the *Shigella dysenteriae* .

Hemanta Koley MSc, PhD, Scientist, Bacteriology
National Institute of Cholera and Enteric Diseases, Kolkata, India

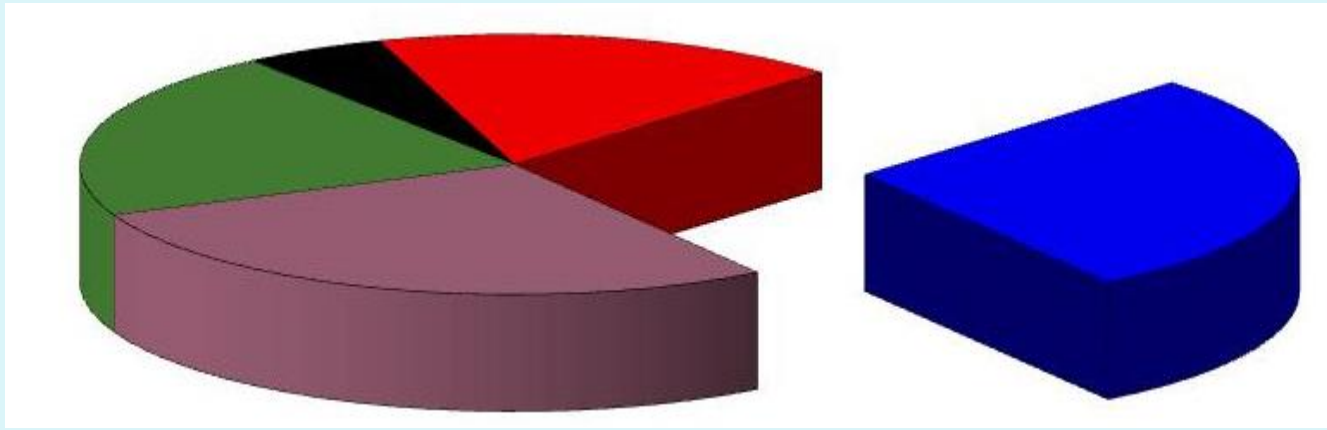
Enteric Pathogens of Global Importance

2.9 Million deaths/year

Cholera
(**150,000** deaths)

ETEC diarrhea
(500,000 deaths)

Typhoid fever
(600,000 deaths)



Rotavirus diarrhea
(**800,000** deaths)

Shigellosis
(800,000 deaths)

WHO Global burden of diseases/ update 2008,WHO/UNICEF

Isolation of enteric pathogens from ID Hospital, Kolkata

(Nov 2007 to Oct 2015)

Pathogen	Age <5 years				Age ≥ 5 yr (n=1871)	All Age Group (n=2519)
	0 - 11 month (n=245)	12 - 23 month (n=227)	24 - 59 month (n=176)	Total Age <5 yr (n=648)		
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Bacteria						
Vibrio cholerae O1	22(9)	34(15)	50(28.4)	106(16.4)	548(29.3)	654(26)
Vibrio cholerae O139	0	0	0	0	2(0.1)	2(0.1)
Vibrio cholerae Non O1 Non O139	2(0.8)	1(0.4)	1(0.6)	4(0.6)	51(2.7)	55(2.2)
V. parahaemolyticus	1(0.4)	0	2(1.1)	3(0.5)	71(3.8)	74(2.9)
Vibrio fluvialis	3(1.2)	7(3.1)	1(0.6)	11(1.7)	44(2.4)	55(2.2)
Aeromonas spp.	1(0.4)	2(0.9)	1(0.6)	4(0.6)	21(1.1)	25(1)
Campylobacter jejuni	18(7.3)	22(9.7)	20(11.4)	60(9.3)	58(3.1)	118(4.7)
C. coli	1(0.4)	0	1(0.6)	2(0.3)	20(1.1)	22(0.9)
Shigellae	**8(3.3)**	**21(9.3)**	**22(12.5)**	**51(7.9)**	**103(5.5)**	**154(6.1)**
Salmonella	0	1(0.4)	1(0.6)	2(0.3)	21(1.1)	23(0.9)
EPEC	11(4.5)	8(3.5)	2(1.1)	21(3.2)	24(1.3)	45(1.8)
ETEC Group	9(3.7)	13(5.7)	5(2.8)	27(4.2)	87(4.6)	114(4.5)
EAEC	32(13.1)	28(12.3)	18(10.2)	78(12)	81(4.3)	159(6.3)
Virus						
Rotavirus	115(46.9)	124(54.6)	39(22.2)	278(42.9)	124(6.6)	402(16)
Adenovirus	24(9.8)	22(9.7)	8(4.5)	54(8.3)	44(2.4)	98(3.9)
Norovirus G1	3(1.2)	5(2.2)	3(1.7)	11(1.7)	21(1.1)	32(1.3)
Norovirus G2	10(4.1)	5(2.2)	2(1.1)	17(2.6)	25(1.3)	42(1.7)
Sapovirus	10(4.1)	4(1.8)	4(2.3)	18(2.8)	13(0.7)	31(1.2)
Astrovirus	5(2)	7(3.1)	5(2.8)	17(2.6)	38(2)	55(2.2)
Parasite						
Blastocystis hominis	0	0	0	0	11(0.6)	11(0.4)
Entamaeba histolytica	8(3.3)	13(5.7)	5(2.8)	26(4)	56(3)	82(3.3)
Giardia lamblia	25(10.2)	34(15)	33(18.8)	92(14.2)	189(10.1)	281(11.2)
Cryptosporidium spp.	37(15.1)	22(9.7)	12(6.8)	71(11)	87(4.6)	158(6.3)

Prevention

Sanitation

Developing country like India, the sanitation problem is acute



Rehydration Therapy

Rehydration therapy is not applicable to the invasive diarrhea.

Antibiotics

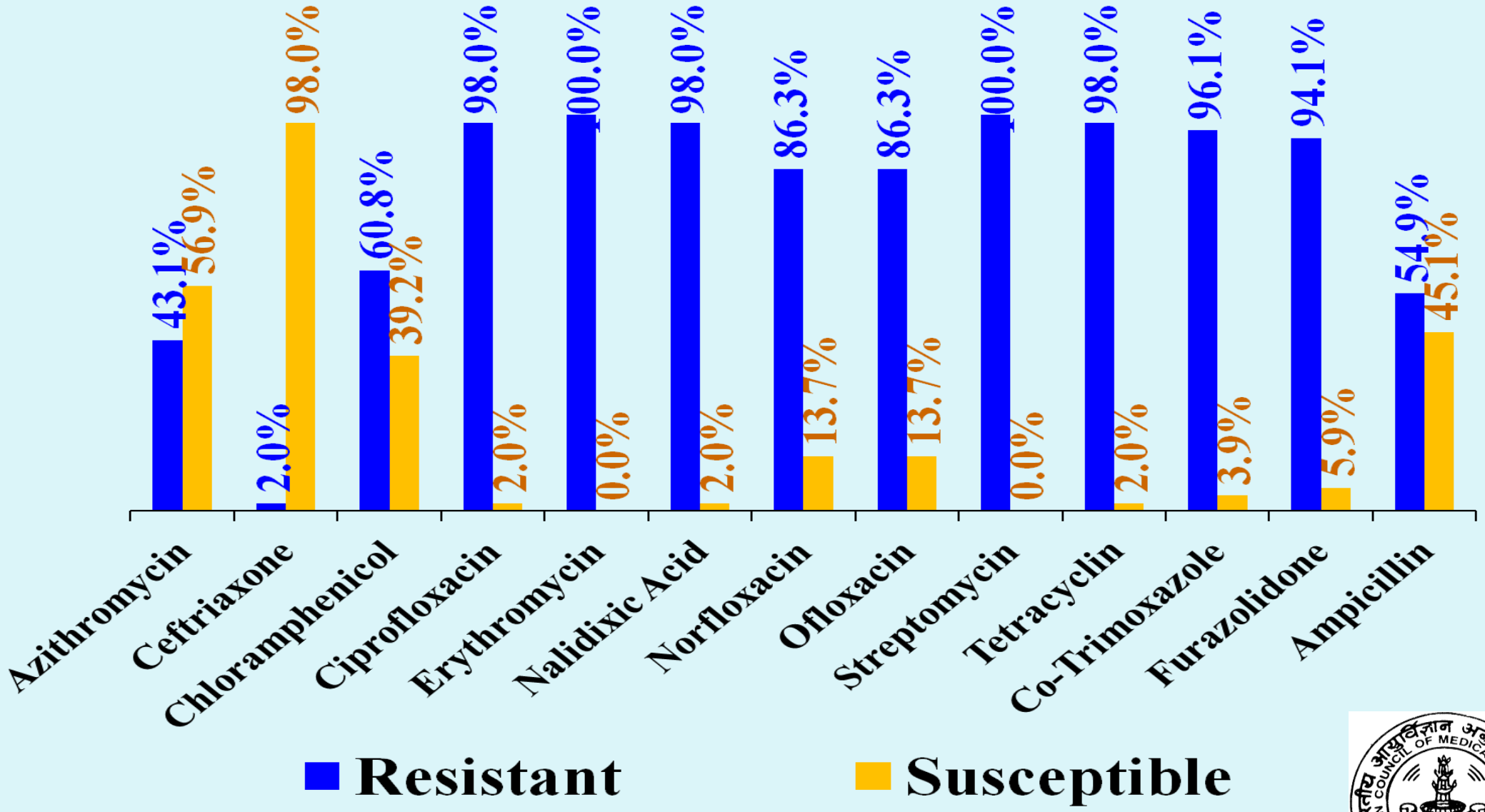
Global emergence of drug resistance, the choice of antimicrobial agents for treating shigellosis is limited.

Vaccine

For more than 50 years, scientists have been trying to come up with suitable candidate vaccines against enteric diseases.

Hemanta Koley MSc, PhD, Scientist, Bacteriology National Institute of Cholera and Enteric Diseases, Kolkata, India

Antimicrobial resistance of Shigella during 2010-15



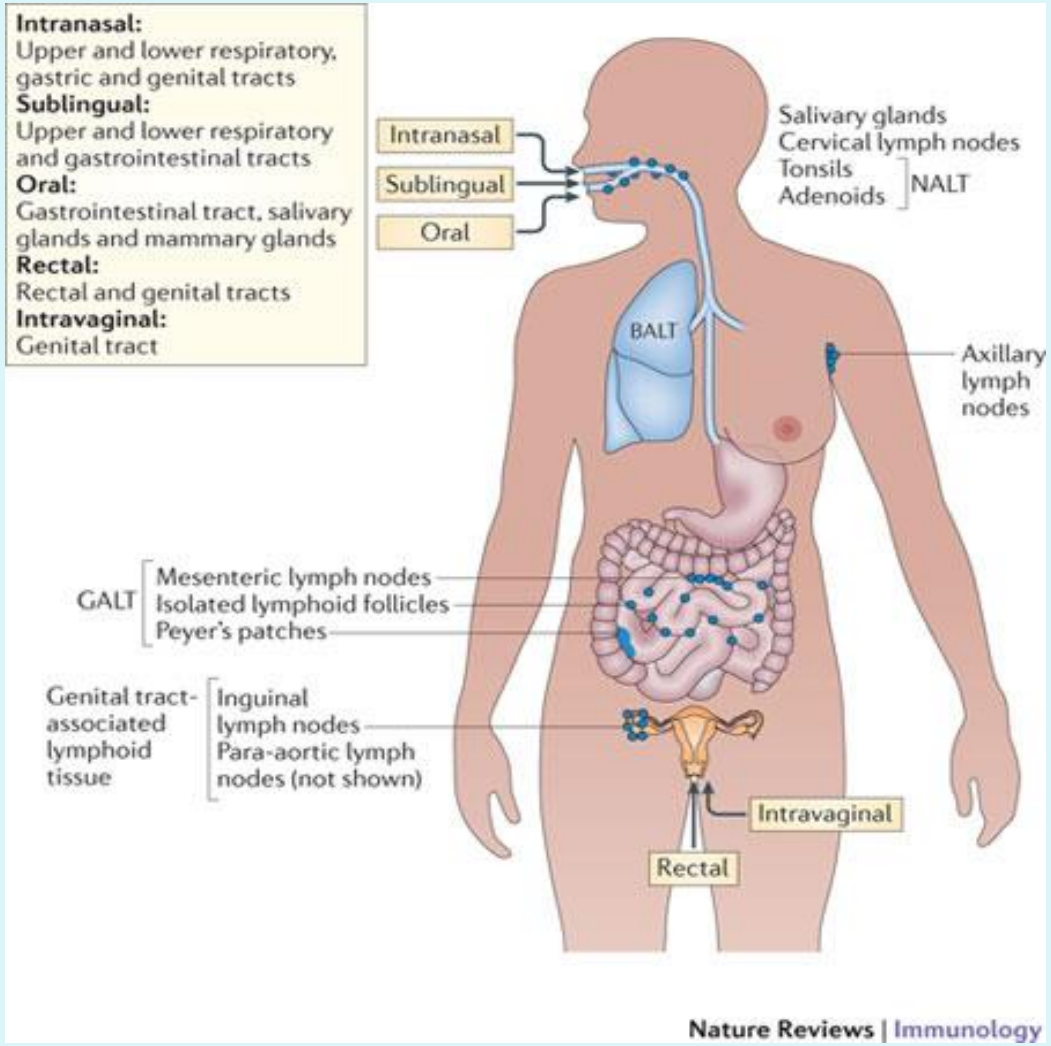
Hemanta Koley MSc, PhD, Scientist, Bacteriology
National Institute of Cholera and Enteric Diseases, Kolkata, India



Vaccination will be the ultimate solution

Hemanta Koley MSc, PhD, Scientist, Bacteriology National Institute of Cholera and Enteric Diseases, Kolkata, India

Routes for human vaccine administration



Oral route of vaccination is the best for the gastrointestinal diseases.

Hemanta Koley MSc, PhD, Scientist, Bacteriology National Institute of Cholera and Enteric Diseases, Kolkata, India



✓ To prepare a unique vaccine formulation different from the candidate vaccines like conjugate, live attenuated, whole cell or ribosomal preparation

Outer Membrane Vesicle: A Novel Particle of Next Generation Vaccine

WHAT are OMVs?

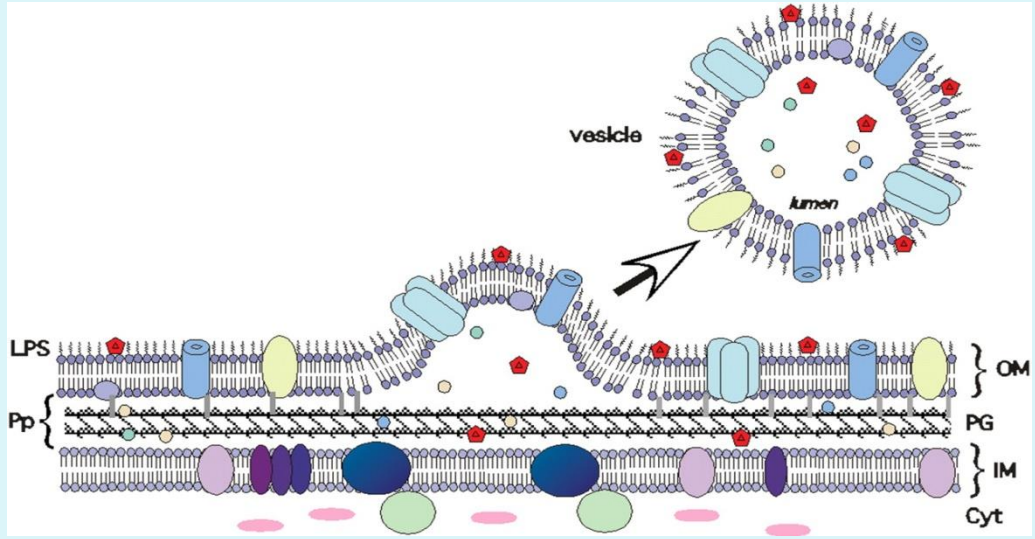
- Most of gram-negative bacteria secrete outer membrane vesicles (OMVs) into the extracellular environment during growth.

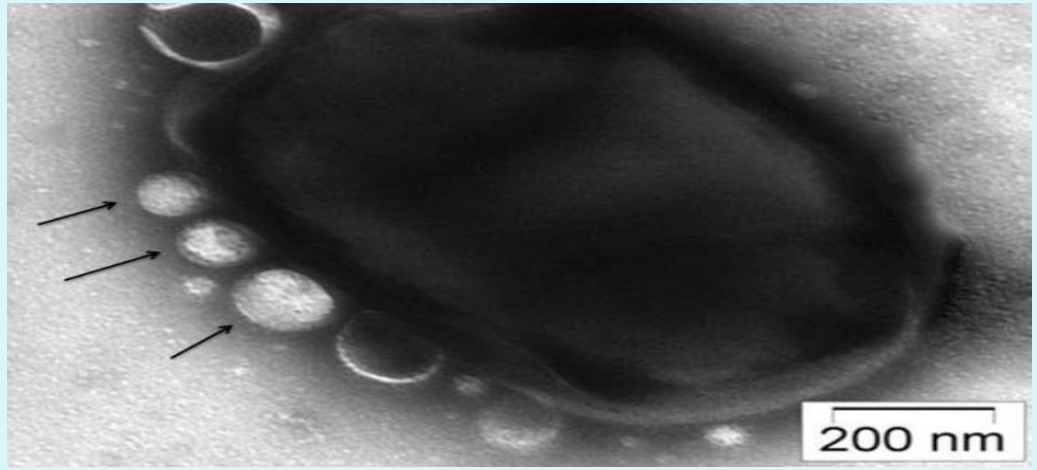
- Spheroid particles of a heterogeneous size (10 to 300 nm in diameter) .

- As because of OMVs originate directly from the plasma membrane of the cell, it reflects the outer membrane (OM) composition, containing LPS, glycerophospholipids, and OM proteins as well as enclosed periplasmic components .

- Play role in interbacterial Interactions. Immunomodulatory activities.

- Non living ; do not have the possibility of reversion like attenuated vaccines.

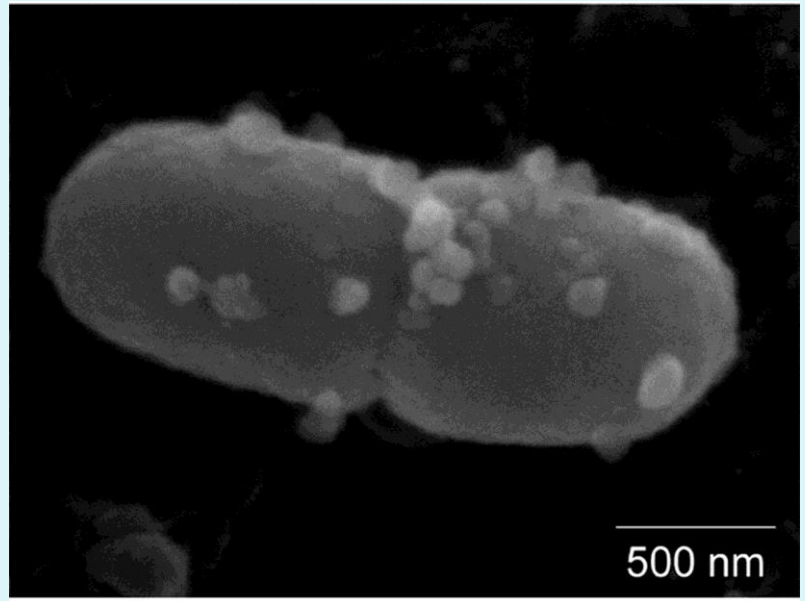


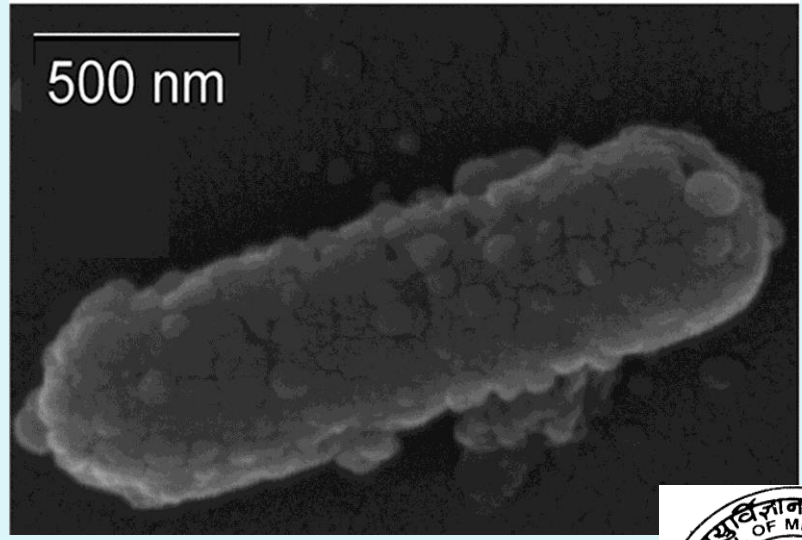


Hemanta Koley MSc, PhD, Scientist D, Bacteriology National Institute of Cholera and Enteric Diseases, Kolkata, India

OUTER MEMBRANE VESICLES (OMVs) as a VACCINE CANDIDATE: A new hope for vaccine study

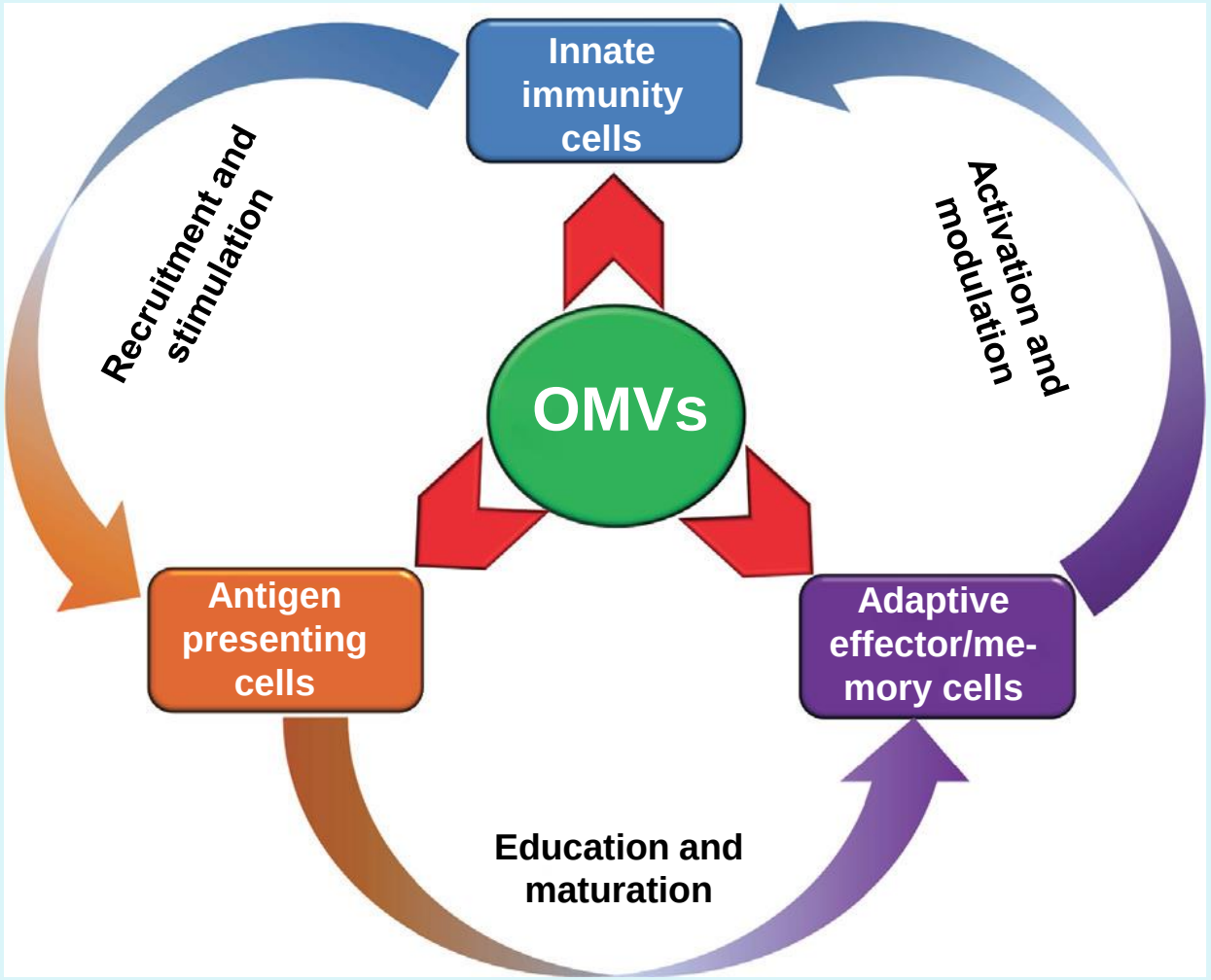






Ellis T N , and Kuehn M J Microbiol. Mol. B
2010;74:81-94

Effecter arms of new generation OMVs vaccines

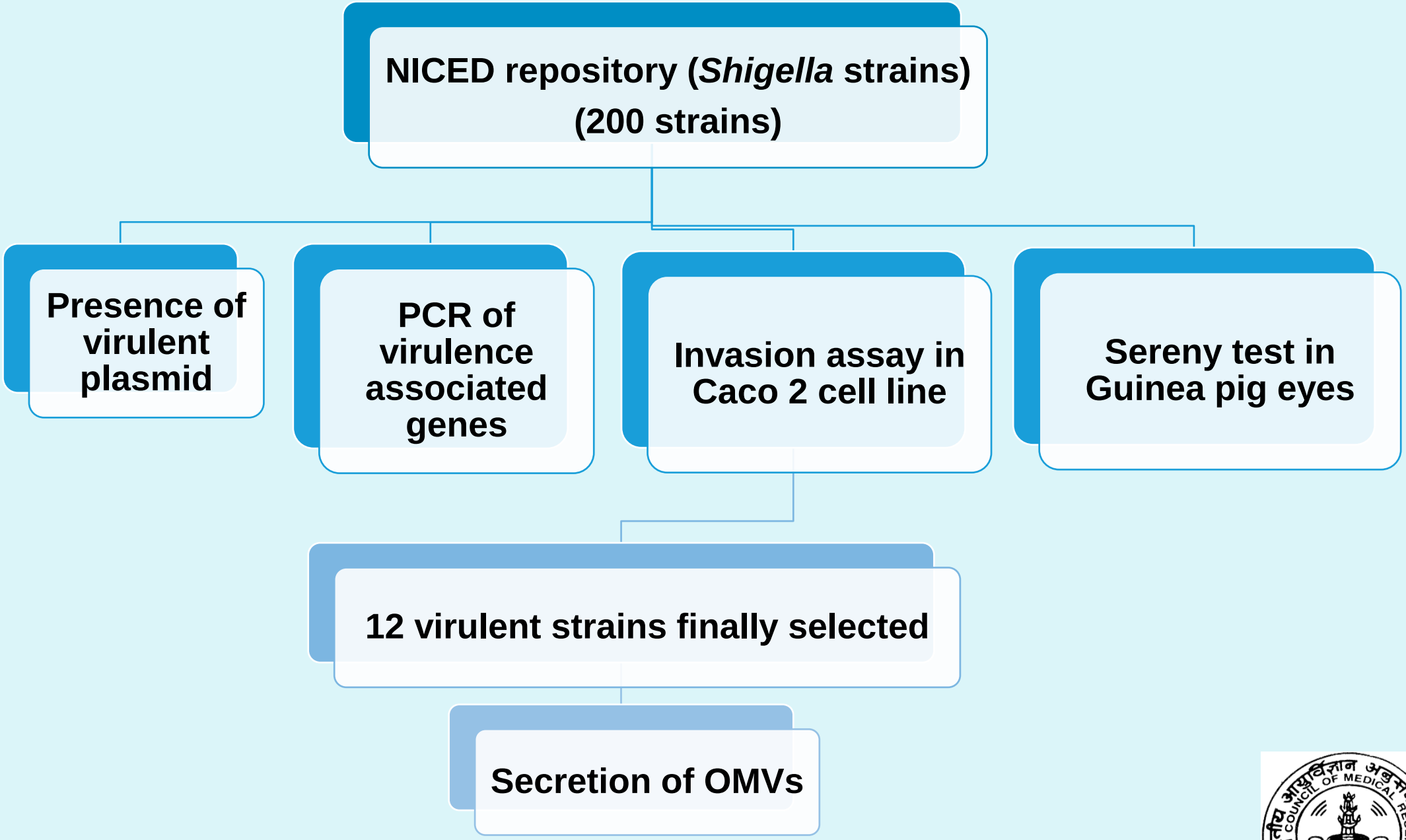


OMVs can induce humoral and adaptive immunity and thus serve long term protection.

OBJECTIVES OF MY STUDY

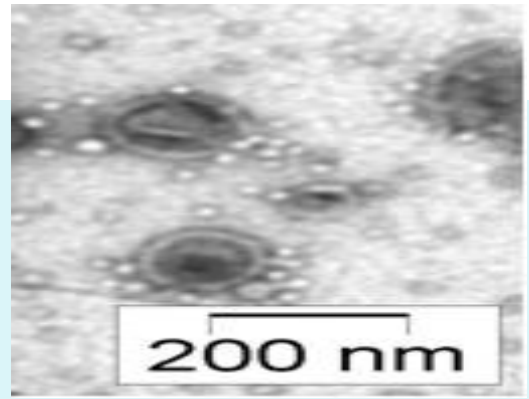
1. Preparation of OMVs immunogen from different serotypes of *Shigella sp.*

2. To understand safety, immunogenicity and protective efficacy of OMVs against 50 heterologous *Shigella sp.* in animal model.


NICED repository (*Shigella* strains) (200 strains)
Presence of virulent plasmid
PCR of virulence associated genes
Invasion assay in Caco 2 cell line
Sereny test in Guinea pig eyes
12 virulent strains finally selected
Secretion of OMVs

Preparation of outer membrane vesicles (OMVs)





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

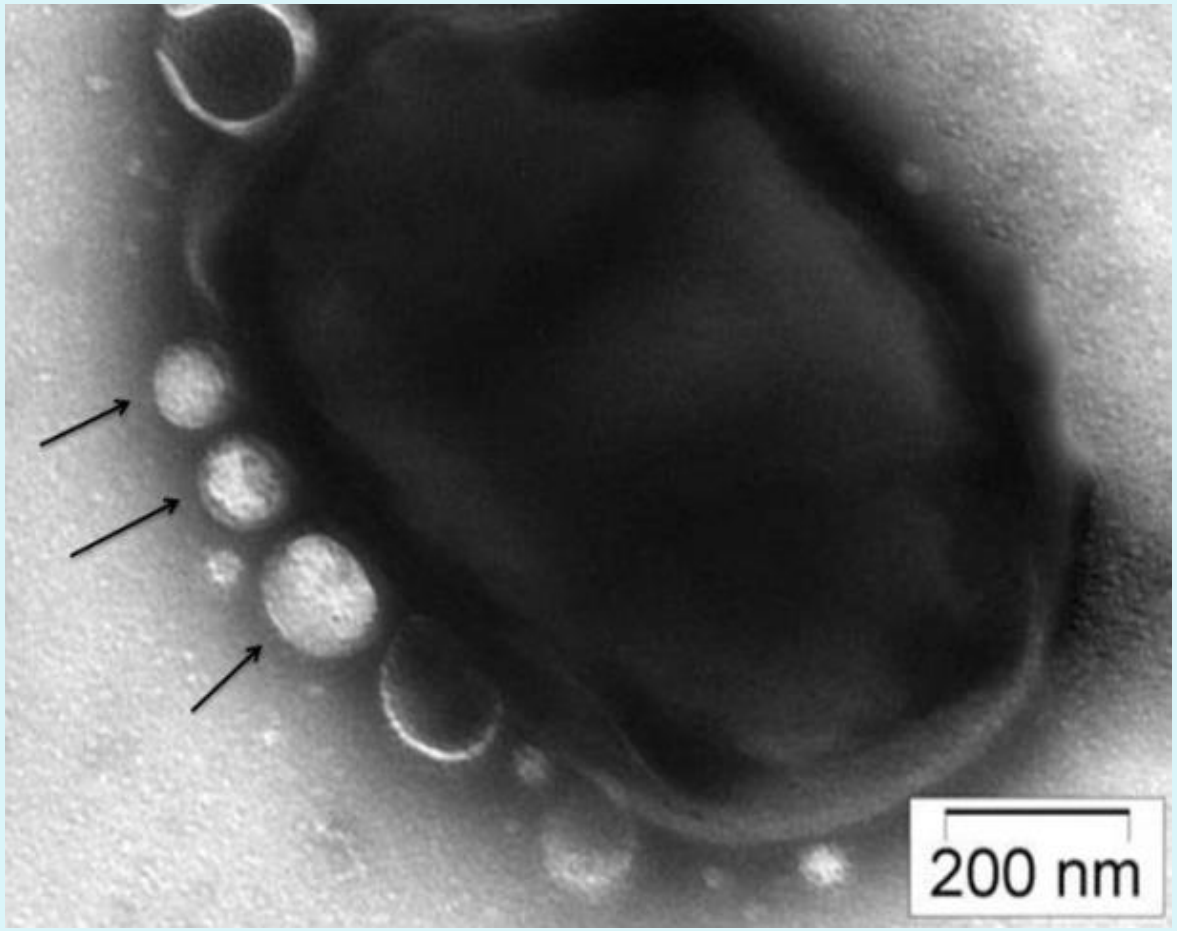
Supernatant was passed through 0.45 and 0.22µm Millipore filter twice.

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 assa

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.

Selection of strain for SOMVs formulation



***S. boydii* 4 (BCH612) was selected for OMVs isolation and single serotype vaccine formulation.**

Hemanta Koley MSc, PhD, Scientist, Bacteriology National Institute of Cholera and Enteric Diseases, Kolkata, India

Protective Efficacy study- passive protection model

7 week old adult female mice

- Immunized with the SOMVs



Adult male mice

- Immunized female mice were mated with age matched males



Suckling mice

- Challenged with wild type virulent *Shigella* strains to study the protective efficacy of the SOMVs vaccine formulation



Scheme for oral immunization with SOMVs and subsequent challenge study







1st Immunization

2nd Immunization

3rd Immunization

4th Immunization

Day 84-90
Mating

Challenge
Day 115- 121

Day 0 blood

Day 7 blood

Day 14 blood

Day 21 blood

Day 28 blood

Day 35 blood

Day 56 blood

Day 78 Blood

Day 120 Blood

Hemanta Koley MSc, PhD, Scientist, Bacteriology National Institute of Cholera and Enteric Diseases, Kolkata, India

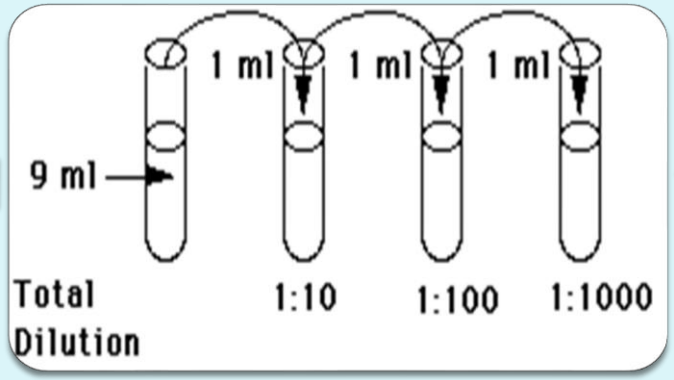
Colonization assay

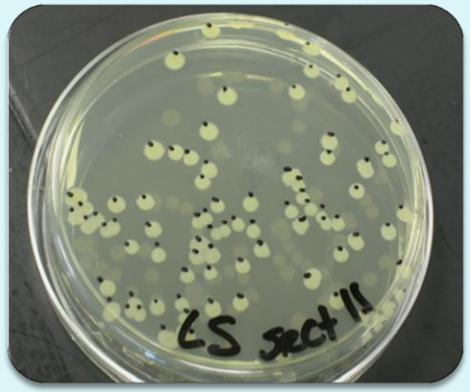




After six hour incubation, neonates were sacrificed and their gut were taken out and homogenized







CFU/gm of intestine of suckling mouse

Hemanta Koley MSc, PhD, Scientist, Bacteriology National Institute of Cholera and Enteric Diseases, Kolkata, India

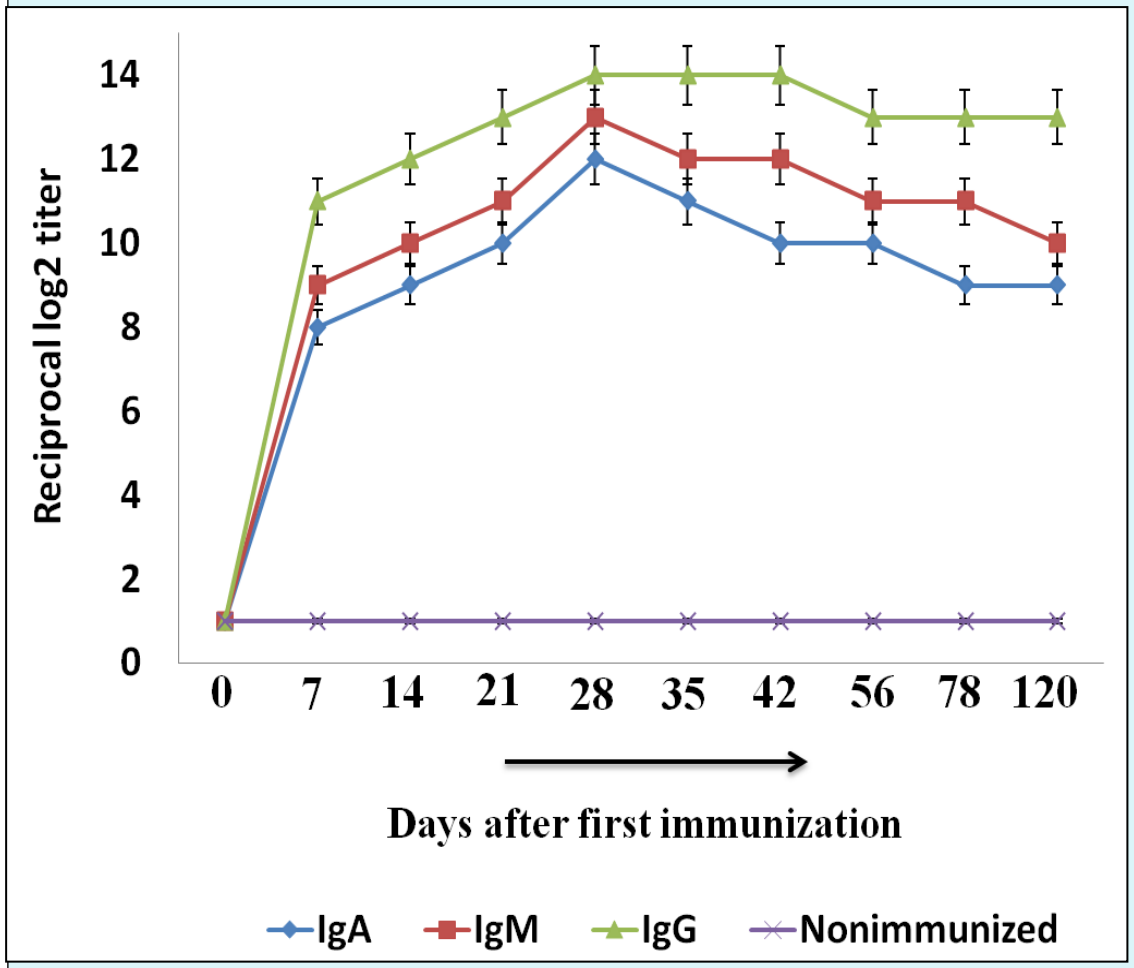
Protection study after SOMVs immunization

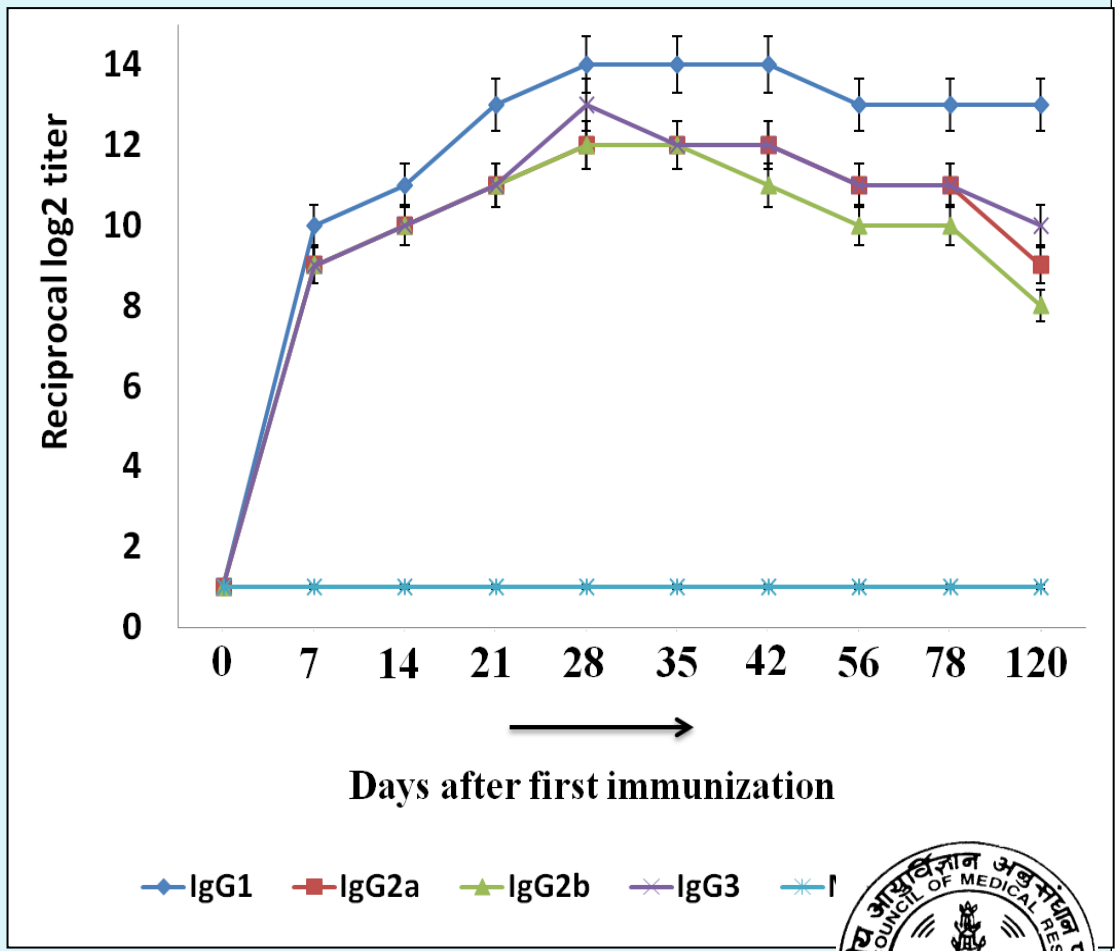
Nature of Study	Challenge strain	Experimental group	No. of animals	% of survival	Protective efficacy (%)
Homologous protection study	*S.boydii* type 4 (BCH612)	Immunized	20	100 (20/20)	**100**
		Nonimmunized	10	20 (2/10)	
Heterologous protection study	*S. dysenteriae* 1 (NT4907)	Immunized	20	85 (17/20)	**81**
		Nonimmunized	10	20 (2/10)	
	S. flexneri 2a (B294)	Immunized	20	80 (16/20)	**75**
		Nonimmunized	10	20 (2/10)	
	S. flexneri 3a (C519)	Immunized	20	70 (14/20)	**77**
		Nonimmunized	10	10 (1/10)	
	S. flexneri 6 (C347)	Immunized	20	80 (16/20)	**71**
		Nonimmunized	10	30 (3/10)	
	S. sonnei (IDH00968)	Immunized	20	80 (16/20)	**75**
		Nonimmunized	10	20 (2/10)	

$$\text{Protective efficacy (Van de verg } et\ al\text{, 1995)} = \frac{(\%\ \text{deaths of controls}) - (\%\ \text{deaths of immunized mice})}{\text{Percent deaths of controls}} \times 100$$

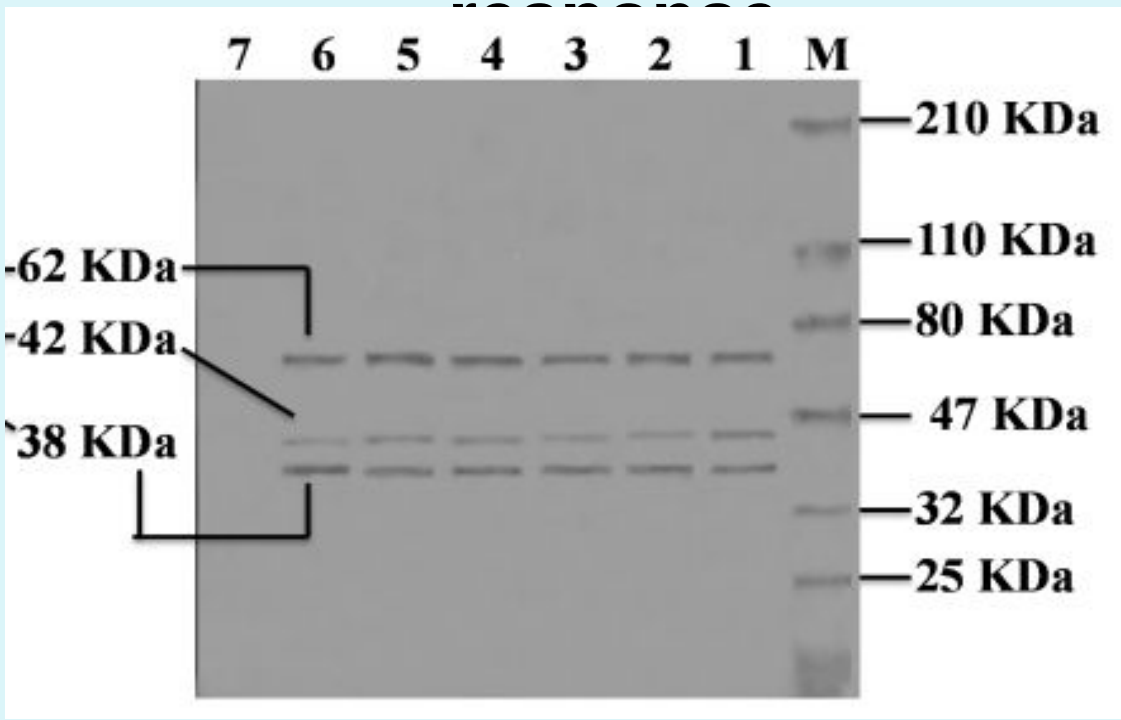
Hemanta Koley MSc, PhD, Scientist, Bacteriology National Institute of Cholera and Enteric Diseases, Kolkata, India

Serum immunoglobulin study of SOMVs



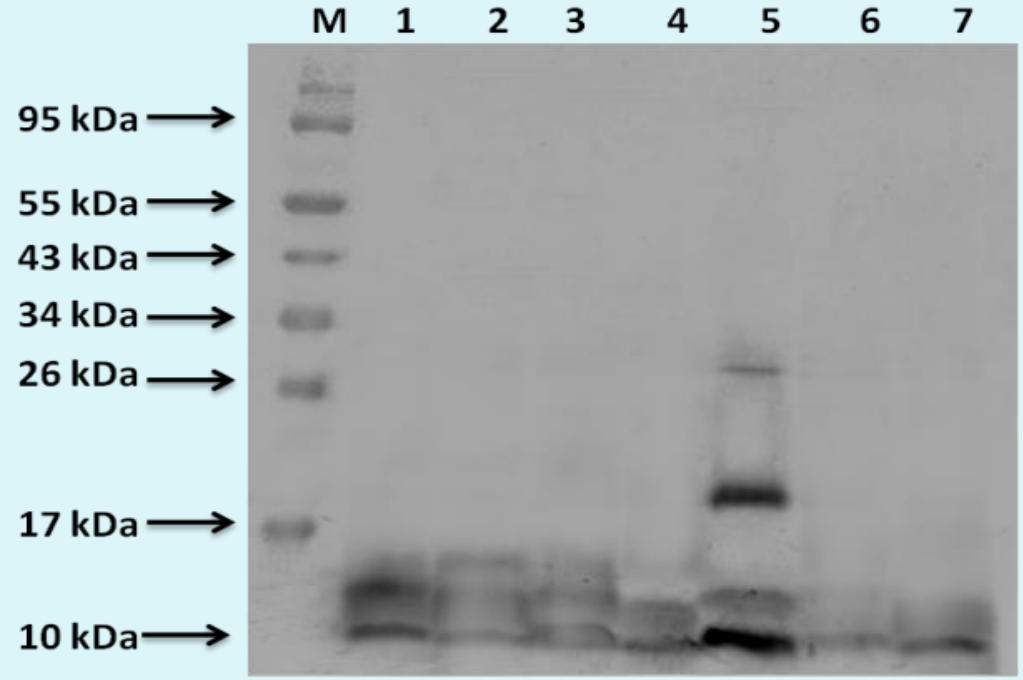


Specificity of the antibody



A

Western blot against whole cell lysates, probed with 28th day anti-SOMVs sera



B

Western blot against LPS, probed with 28th day anti-SOMVs sera

Lane M: protein molecular weight marker (BioRad)
Lane 1: *S. dysenteriae* 1
Lane 2: *S. flexneri* 2a
Lane 3: *S. flexneri* 3a
Lane 4: *S.flexneri* 6
Lane 5: *S. boydii* type 4
Lane 6: *S.sonnei*
Lane 7*: S. flexneri* 1a (avirulent strain)

Hemanta Koley MSc, PhD, Scientist, Bacteriology



- ***Shigella boydii*** **type 4 BCH612 secretes vesicles during growth which are highly immunogenic in adult mice.**
- **IpaB, IpaC and IpaD are probably the most immunogenic proteins of OMVs.**
- **These OMVs has given 100% homologous but 75% heterologous protection in average to the offsprings of immunized females.**

FEMS IMMUNOLOGY & MEDICAL MICROBIOLOGY

Research Paper

Outer membrane vesicles of *Shigella boydii* type 4 induce passive immunity in neonatal mice

Soma Mitra[1], Soumik Barman[1], Dhrubajyoti Nag[1], Ritam Sinha[1], Dhira Rani Saha[2], Hemanta Koley[1,*]

DOI: 10.1111/j.1574-695X.2012.01004.x

© 2012 Federation of European Microbiological Societies. Published by Blackwell Publishing Ltd. All rights reserved

Issue



FEMS Immunology & Medical Microbiology

Accepted Article. These manuscripts have been accepted, but have not been edited or formatted. They will be published at a future date.

Publication date : 2012 Nov; 66(2):240-50.

This is the first report of outer membrane vesicles induced passive protection study in mice model against shigellosis.

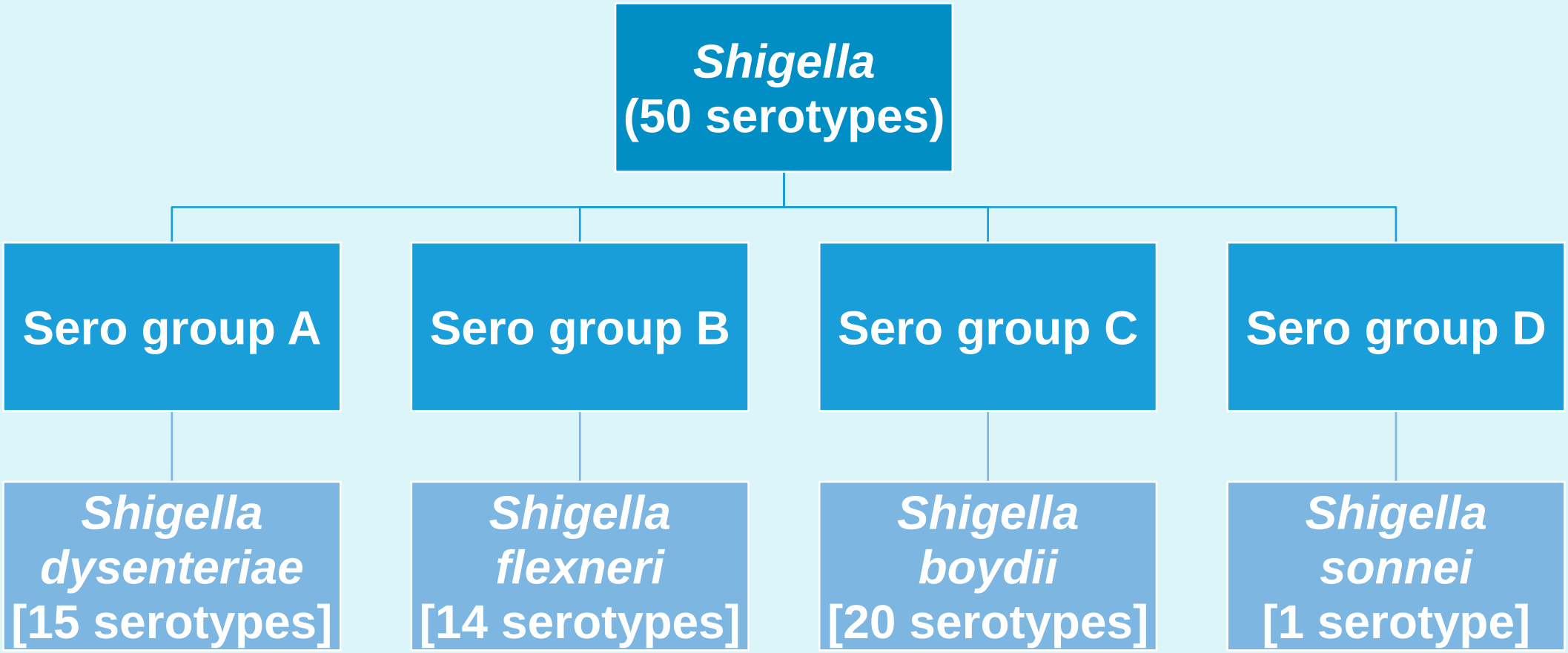


Though 100% homologous protection was quite satisfactory,

75% average heterologous protective efficacy bestowed by the OMVs of *S. boydii* was not at all promising from the purview of disease prevalence and severity.



This is quite obvious if we look at the number of serotypes and subserotypes among *Shigella sp.*
A single serotype vaccine is not a very promising idea



Source: Levine MM et al, .Clinical trials of Shigella vaccines: two steps forward and one step back o hard road. Nat Rev Microbiol. 2007 Jul;5(7):540-53. Review.

Hemanta Koley MSc, PhD, Scientist, Bacteriology National Institute of Cholera and Enteric Diseases, Kolkata, India



<u>Multi-serotype form of vaccine</u> is the future for absolute eradication of shigellosis associated death in young children

Hemanta Koley MSc, PhD, Scientist, Bacteriology National Institute of Cholera and Enteric Diseases, Kolkata, India



OMVs from multiple Shigella strains

Cocktail

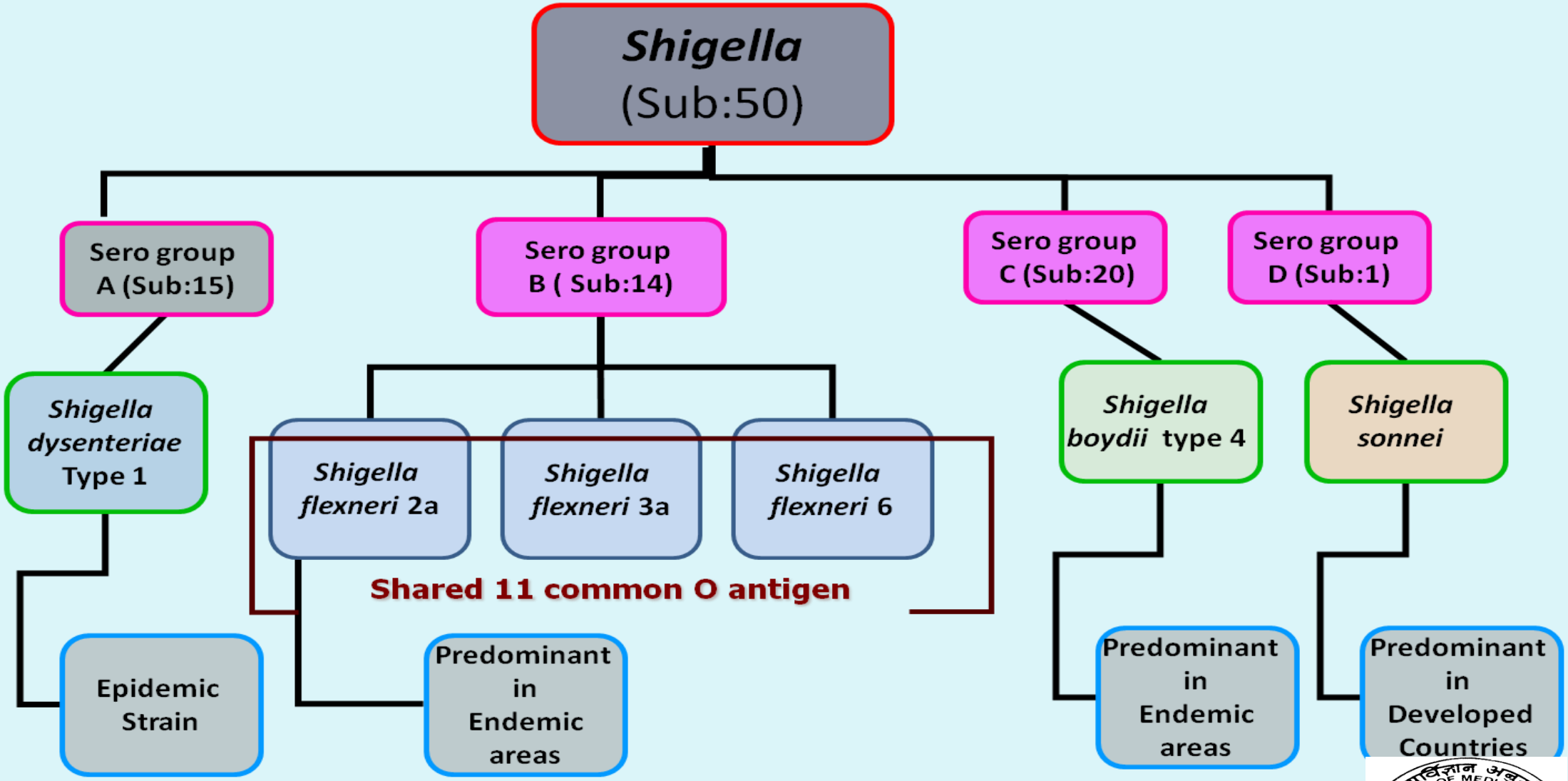
Multi-serotype Outer Membrane Vesicles

Or

MOMVs

Hemanta Koley MSc, PhD, Scientist, Bacteriology National Institute of Cholera and Enteric Diseases, Kolkata, India

Selection of strains: Concept behind MOMVs vaccine formulation



Six components of the MOMVs (hexavalent) vaccine

1. *S. dysenteriae* 1 ∆stx (NT4907)

2. *S. flexneri* 2a (B294)

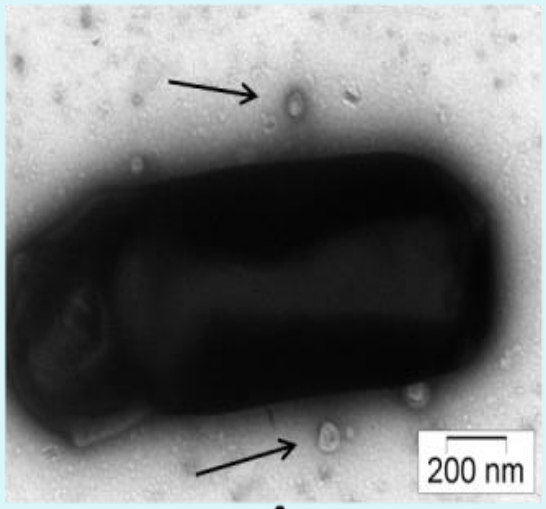
3. *S. flexneri* 3a (C519)

4. *S. flexneri* 6 (C347)

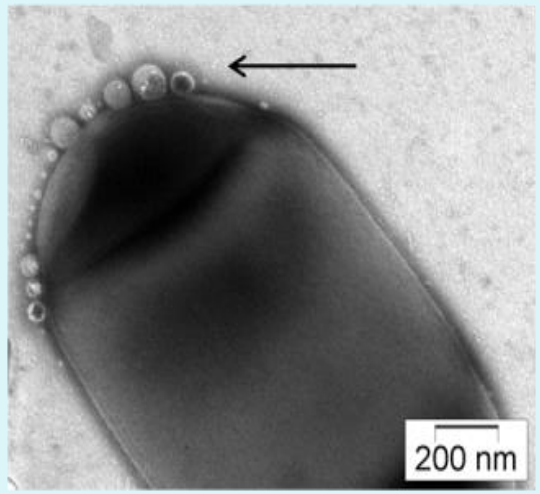
5. *S. boydii* 4 (BCH612)

6. *S. sonnei* (IDH00968)

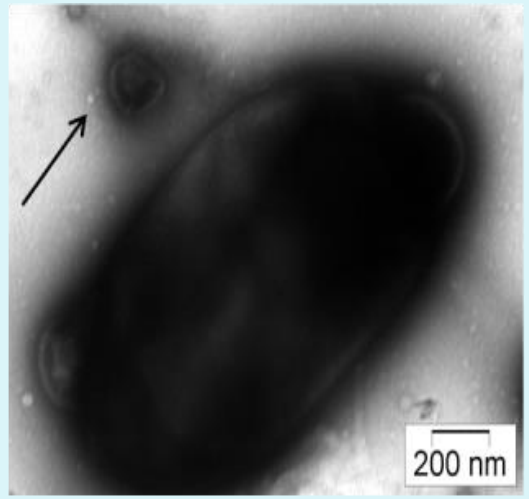
Electron microscopic view of OMVs secreting *Shigella*



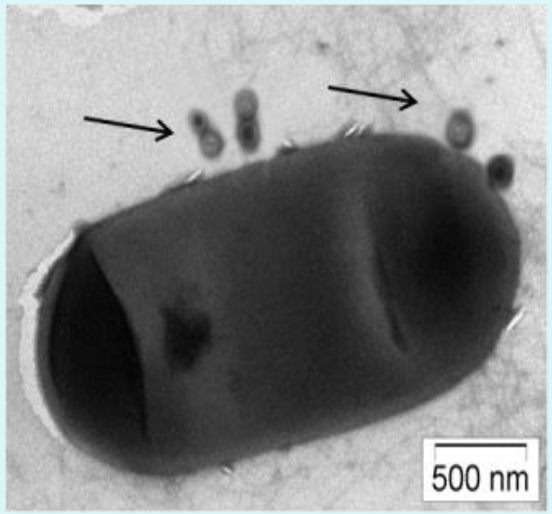
A



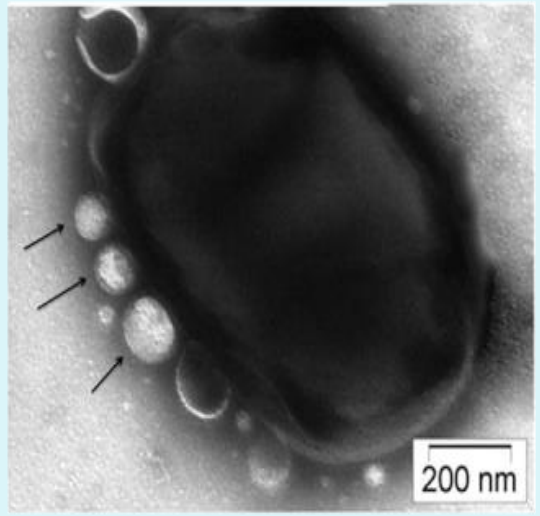
B



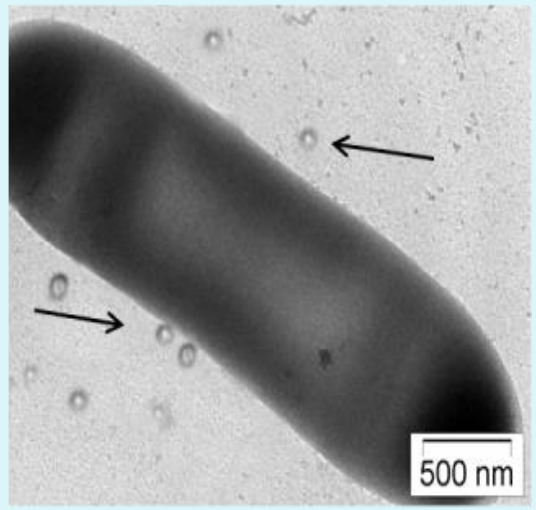
C



D



E



F

A: *S. dysenteriae*1Δstx

B: *S. flexneri* 2a

C: *S. flexneri* 3a

D: *S.flexneri* 6

E: *S. boydii* type 4

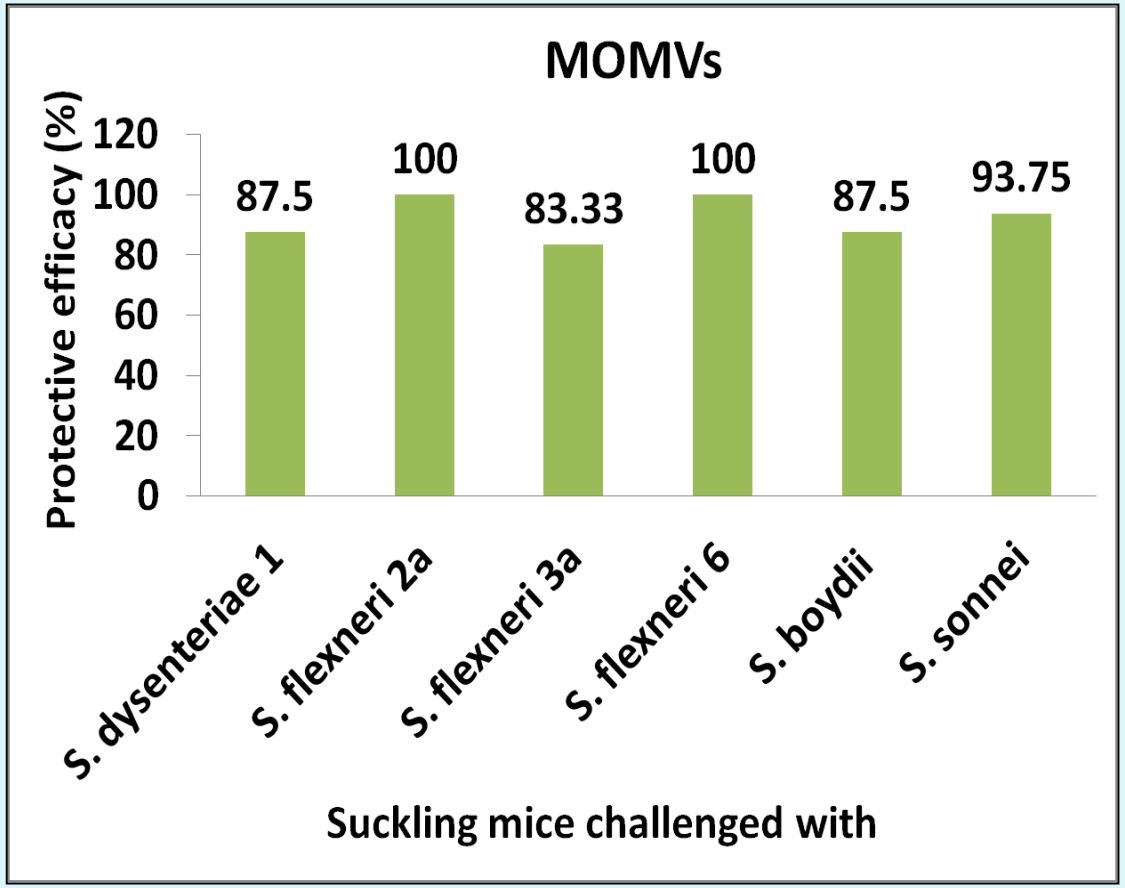
F: *S.sonnei*

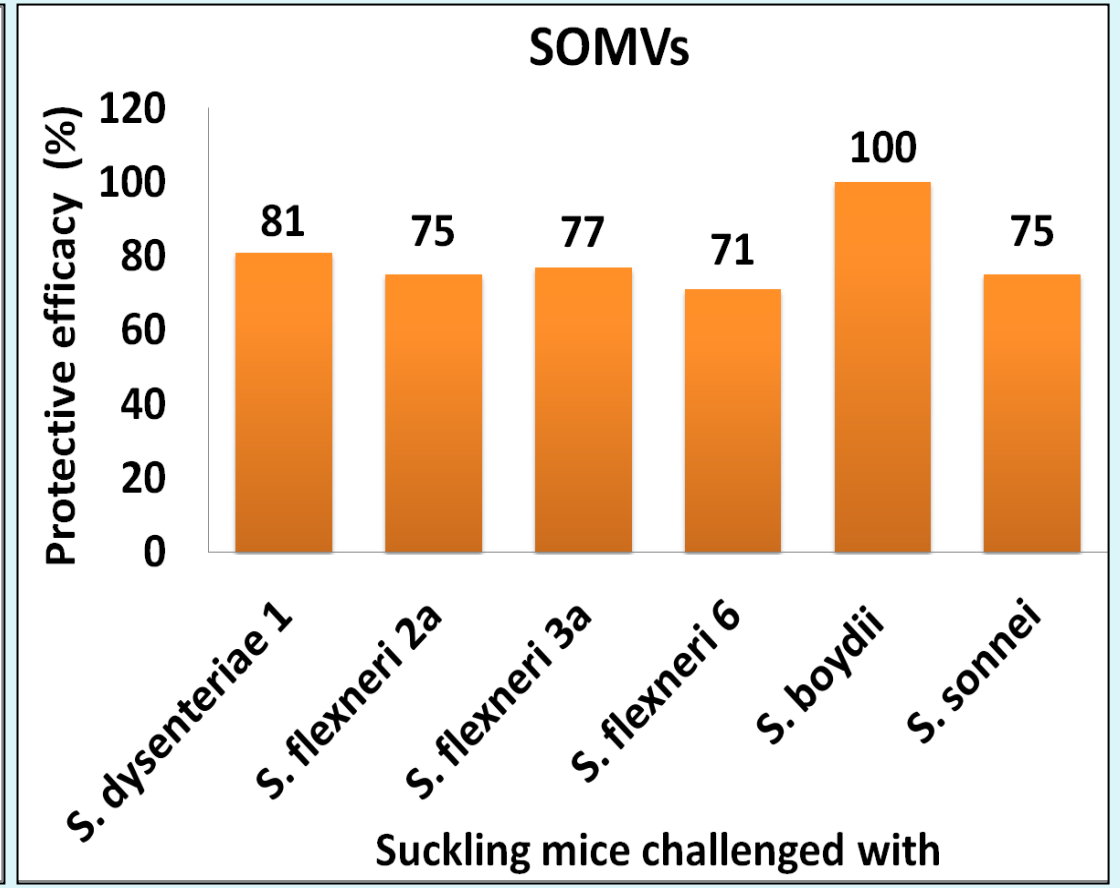
Protective efficacy of MOMVs

Challenged strain	Infectious dose$_{50}$ (ID_{50})	Challenge dose[a]	Experimental group	Experimental neonates	% of survival after challenge	Protective efficacy (%) after challenge
S.dysenteriae 1 (A1)	$2 \pm 0.201 \times 10^8$/ml	$3 \pm 0.336 \times 10^{10}$ /ml	Control	10	20 (2/10)	87.5
			Immunized	20	90 (18/20)	
S. flexneri 2a (NK3809)	$3 \pm 0.251 \times 10^8$/ml	$4 \pm 0.258 \times 10^{10}$ /ml	Control	10	20 (2/10)	100
			Immunized	20	100 (20/20)	
S. flexneri 3a (NK3758)	$3 \pm 0.193 \times 10^8$/ml	$3 \pm 0.101 \times 10^{10}$ /ml	Control	10	10 (1/10)	83.33
			Immunized	20	85 (17/20)	
S. flexneri 6 (NK4025)	$5 \pm 0.211 \times 10^8$ /ml	$5 \pm 0.46 \times 10^{10}$ /ml	Control	10	30 (3/10)	100
			Immunized	20	100 (20/20)	
S. boydii 2 (NK4023)	$4 \pm 0.190 \times 10^8$/ml	$5 \pm 0.64 \times 10^{10}$ /ml	Control	10	20 (2/10)	87.5
			Immunized	20	90 (18/20)	
S. sonnei (NK3918)	$2 \pm 0.134 \times 10^8$/ml	$2 \pm 0.167 \times 10^{10}$ /ml	Control	10	20 (2/10)	93.75
			Immunized	20	95 (18/20)	

Hemanta Koley MSc, PhD, Scientist, Bacteriology

Comparison of protective efficacy





$$\text{Protective efficacy (Van de verg } et\ al, 1995) = \frac{(\%\ \text{deaths of controls}) - (\%\ \text{deaths of immunized mice})}{\text{Percent deaths of controls}} \times 100$$

Hemanta Koley MSc, PhD, Scientist, Bacteriology National Institute of Cholera and Enteric Diseases, Kolkata, India

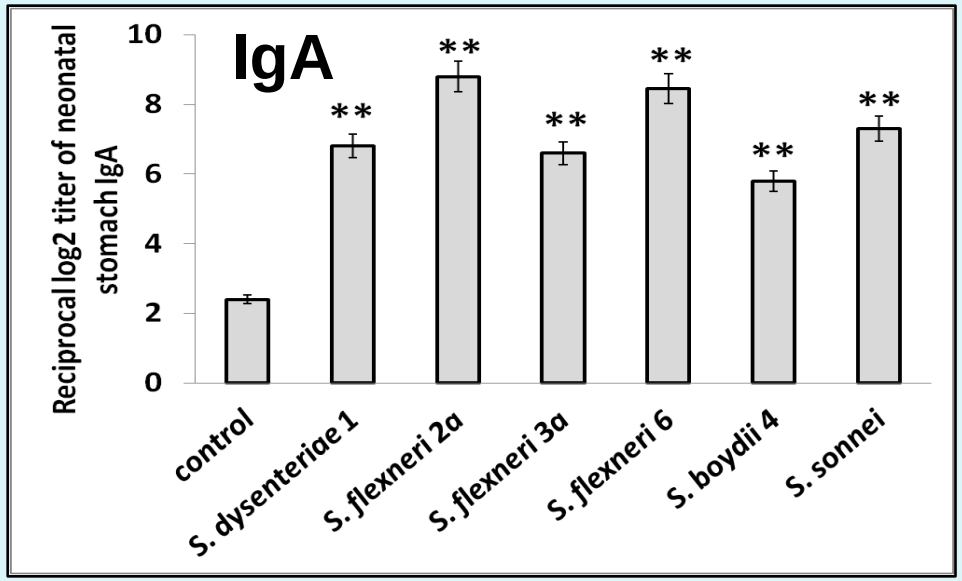
Mother’s milk: the main source of passive protection

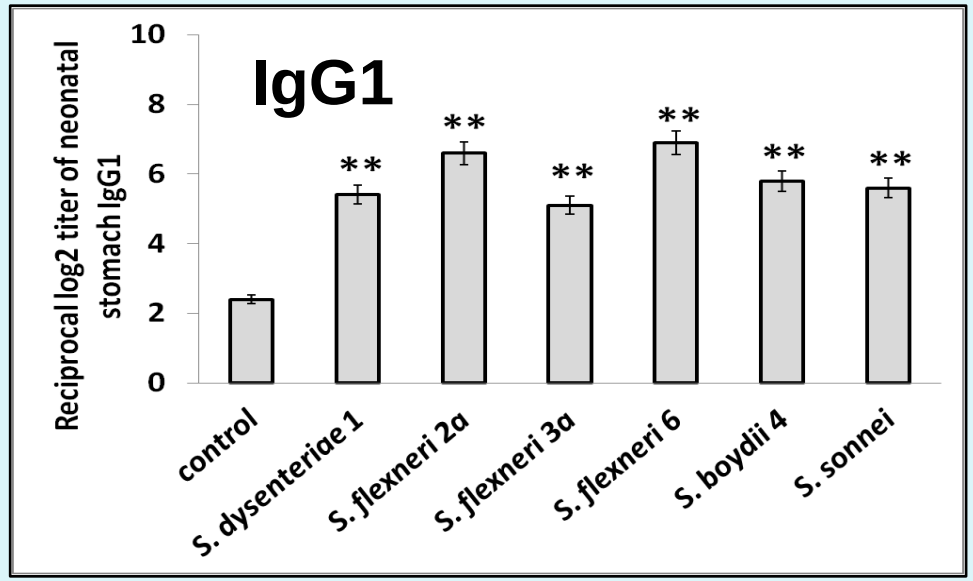


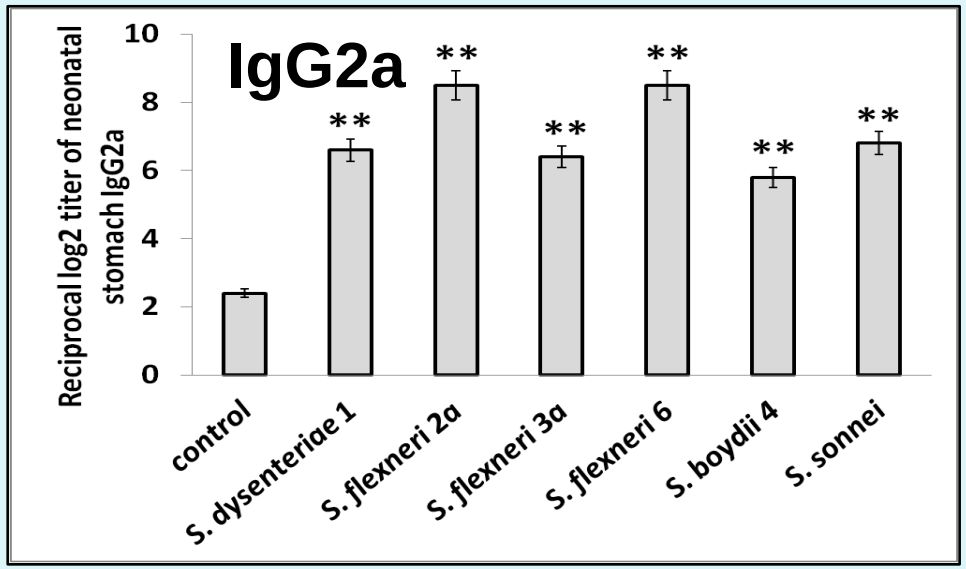
- Neonatal gut mainly contain milk from their mother.

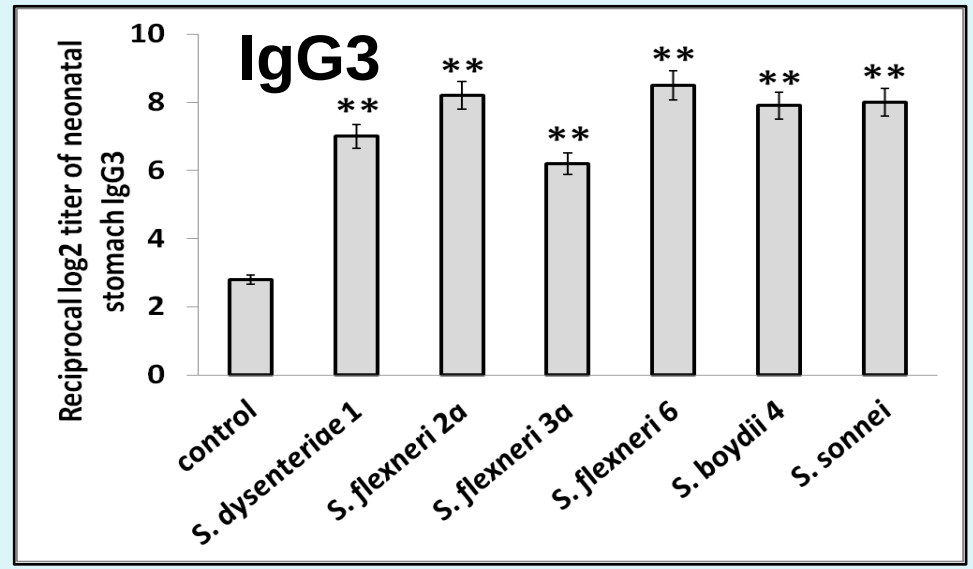
- Immunoglobulins in the gut of the neonatal mice from immunized dams were studied by ELISA

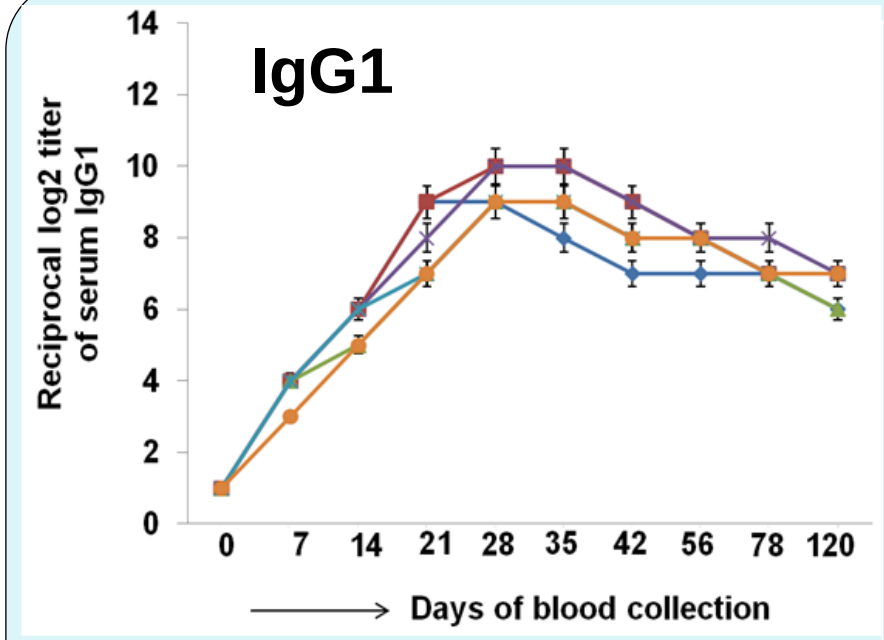
Hemanta Koley MSc, PhD, Scientist, Bacteriology National Institute of Cholera and Enteric Diseases, Kolkata, India

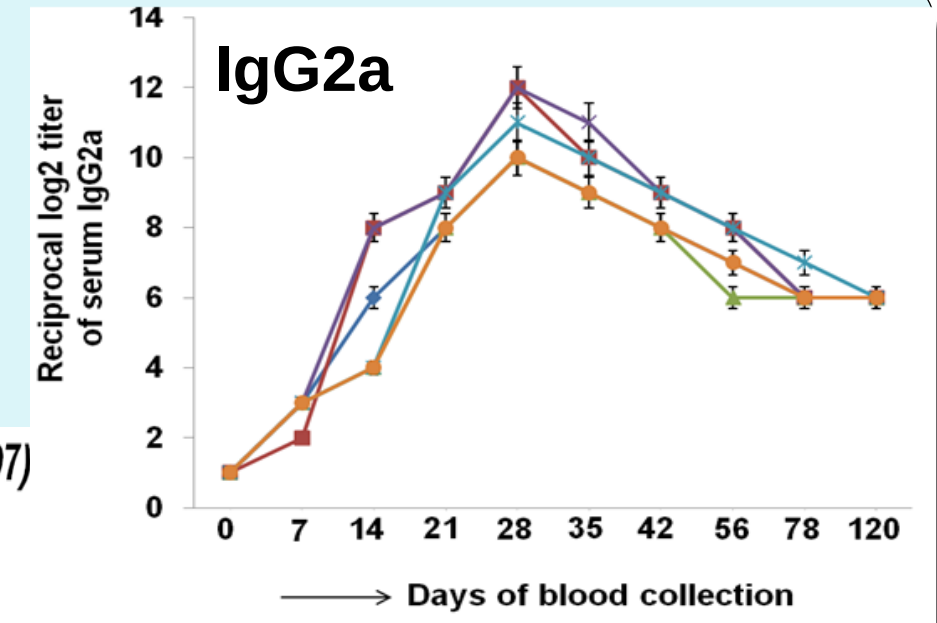

IgA
Reciprocal log2 titer of neonatal stomach IgA
10
8
6
4
2
0
**
control
S. dysenteriae 1
S. flexneri 2a
S. flexneri 3a
S. flexneri 6
S. boydii 4
S. sonnei

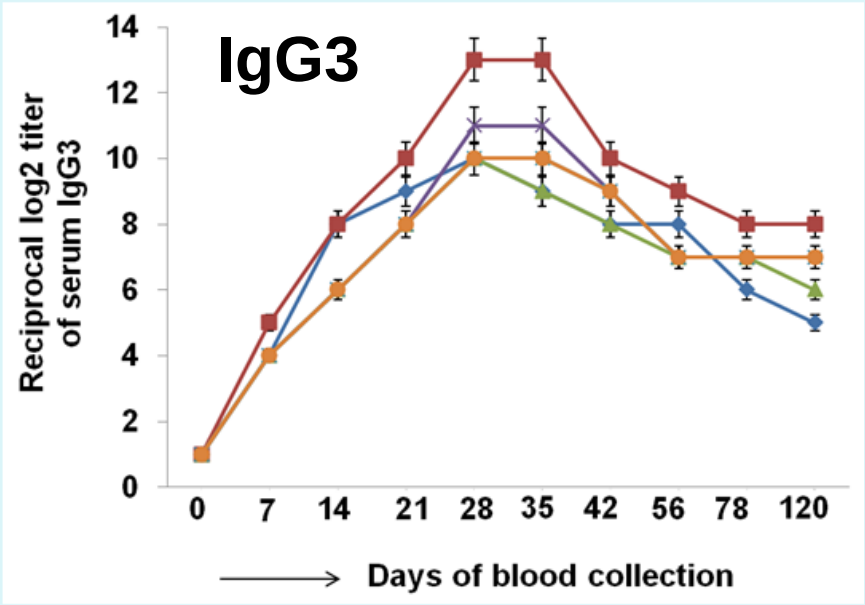

IgG1
Reciprocal log2 titer of neonatal stomach IgG1
10
8
6
4
2
0
**
control
S. dysenteriae 1
S. flexneri 2a
S. flexneri 3a
S. flexneri 6
S. boydii 4
S. sonnei


IgG2a
Reciprocal log2 titer of neonatal stomach IgG2a
10
8
6
4
2
0
**
control
S. dysenteriae 1
S. flexneri 2a
S. flexneri 3a
S. flexneri 6
S. boydii 4
S. sonnei

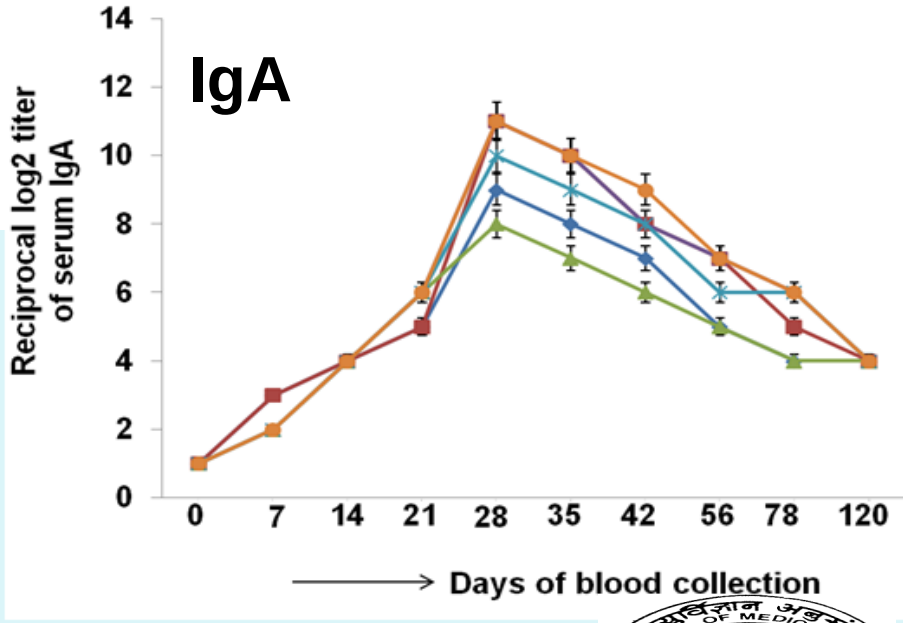

IgG3
Reciprocal log2 titer of neonatal stomach IgG3
10
8
6
4
2
0
**
control
S. dysenteriae 1
S. flexneri 2a
S. flexneri 3a
S. flexneri 6
S. boydii 4
S. sonnei



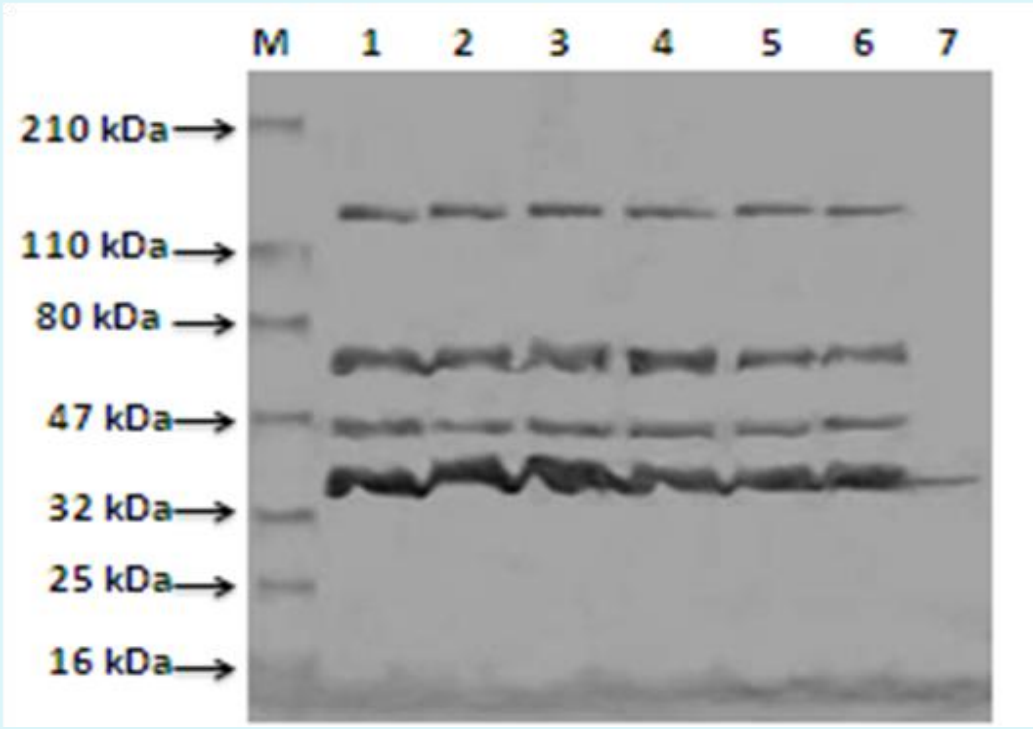




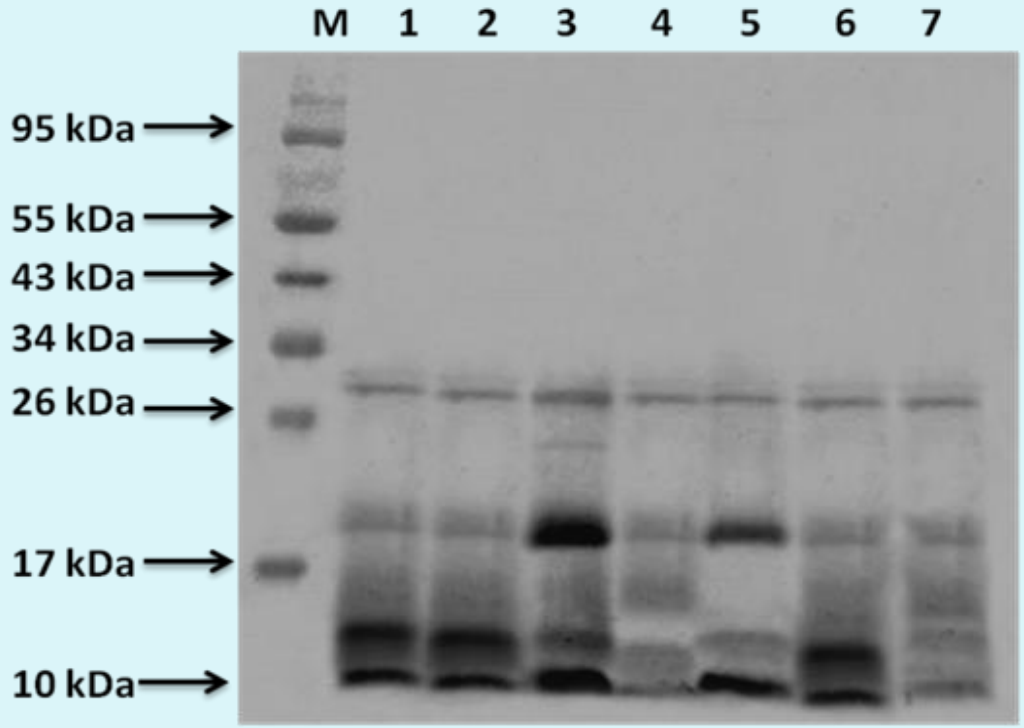
S. dysenteriae1 (NT4907)
S. flexneri 2a (B294)
S. flexneri 3a (C519)
S. flexneri 6 (C347)
S. boydii 4 (BCH612)
S. sonnei (IDH00968)



Western blot against whole cell lysates (WCL) and LPS: probed with anti-MOMVs sera



WCL



LPS

Lane M: protein molecular weight marker (BioRad)

Lane 1: ***S. dysenteriae* 1** **Lane 2: *S. flexneri* 2a**

Lane 3: *S. flexneri* 3a **Lane 4: *S.flexneri* 6**

Lane 5: *S. boydii* type 4 **Lane 6: *S.sonnei***

Lane 7*: S. flexneri* 1a (avirulent strain)



- The protective nature of various proteins along with the natural adjuvant LPS, have made our newly developed multi-serotype (hexavalent) OMVs formulation an ultimate broad spectrum non-living vaccine candidate.

- Multi-serotype (hexavalent) OMVs formulation without any artificial adjuvant conferred long term passive protection to neonatal mice against shigellosis.

- It carries two important qualities of an ideal vaccine: broad spectrum immunogenicity and low reactogenicity.

- It could be exploited for the development of a novel non-living vaccine against human shigellosis in near future.

Vaccine 31 (2013) 3163–3173

Contents lists available at SciVerse ScienceDirect

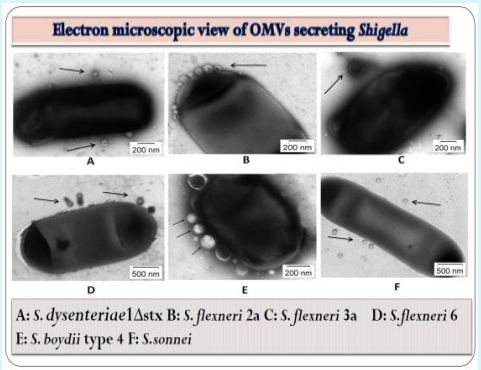
Vaccine

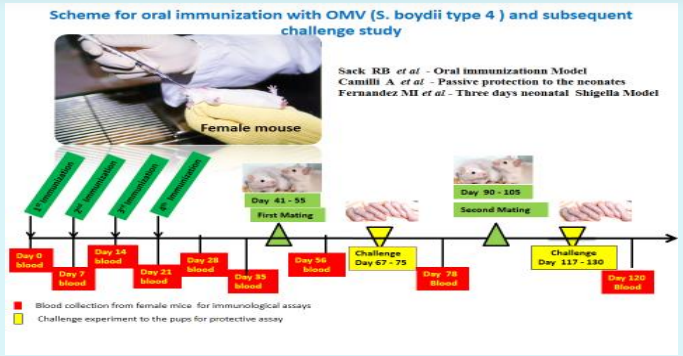
journal homepage: www.elsevier.com/locate/vaccine

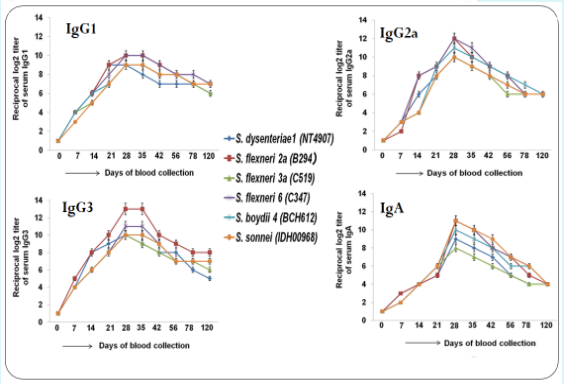
Multi-serotype outer membrane vesicles of *Shigellae* confer passive protection to the neonatal mice against shigellosis

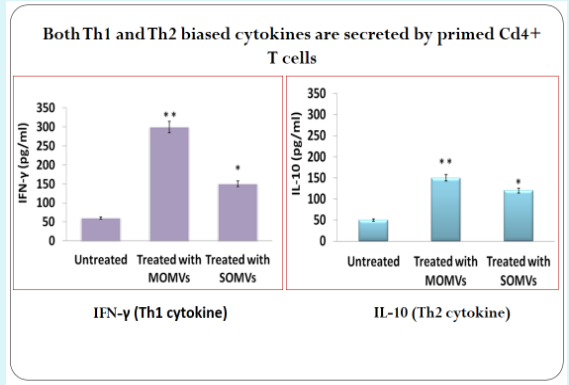
Soma Mitra [a], Manoj K. Chakrabarti [b], Hemanta Koley [a,*]

[a] Division of Bacteriology, National Institute of Cholera and Enteric Diseases, P-33, C.I.T. Road, Scheme XM, Beliaghata, Kolkata 700010, India
[b] Division of Pathophysiology, National Institute of Cholera and Enteric Diseases, P-33, C.I.T. Road, Scheme XM, Beliaghata, Kolkata 700010, India









Highlights

- Formulation of multi-serotype OMVs based immunogen against shigellosis.
- Mixed Th1/Th2 cell mediated immune response without any adjuvant, in adult mice.
- Up to 80% passive protection was observed in neonatal mice against shigellosis.

This is the first report on the competency of a multi-serotype OMVs based vaccine to confer protective immunity against shigellosis in mice model.

Trials in Vaccinology 4 (2015) 56–60

Contents lists available at ScienceDirect

Trials in Vaccinology

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

Immunomodulatory role of outer membrane vesicles of *Shigella* in mouse model

Soma Mitra, Ritam Sinha, Dhrubajyoti Nag, Hemanta Koley*

Division of Bacteriology, National Institute of Cholera and Enteric Diseases, P-33, C.I.T. Road, Scheme XM, Beliaghata, Kolkata 700010, India

- **OMVs of *Shigella* activate various pro-inflammatory (TNF-a, IL-1b, IL-6, IL-12, IL-18, IFN-γ) and anti-inflammatory (IL-4 and IL-10) cytokine in vivo and in vitro system.**

- **It also up regulated mixed Th1/Th2 cellular response which may induce long term immunity and protective efficacy against Shigellosis.**

Contributed to my present work :

Senior Members



Soma Mitra MSc PhD



Soumik Barman MSc PhD



Nivedita Roy MSc PhD

Senior Students



Dhrubajyoti Nag MSc



Ritam Sinha MSc



Priyadarshini Mukherjee MSc

Junior Students



Debaki Ranjan Howlader MSc



Usasi Bhaumik MSc



Paushali Ghosh M Tech

Need to Wait for Vaccine, why not follow these steps:

The following tips can help prevent *shigella* infection:



- ❑ **Wash hands thoroughly with soap, especially after going to the toilet.**



- ❑ **Supervise hand washing of toddlers and small children after they use the toilet.**

- ❑ **Take care of food and drinking water.**



For more information about shigellosis check with your doctor or health department, or consult the CDC web site:
www.cdc.gov/ncidod/dbmd/diseaseinfo/shigellosisg.htm



National Institute of Cholera and Enteric Diseases

(Indian Council of Medical Research)

P-33 CIT Road, Scheme-XM, Beliaghata,
Kolkata, India
Contact : hemantakoley@hotmail.com

