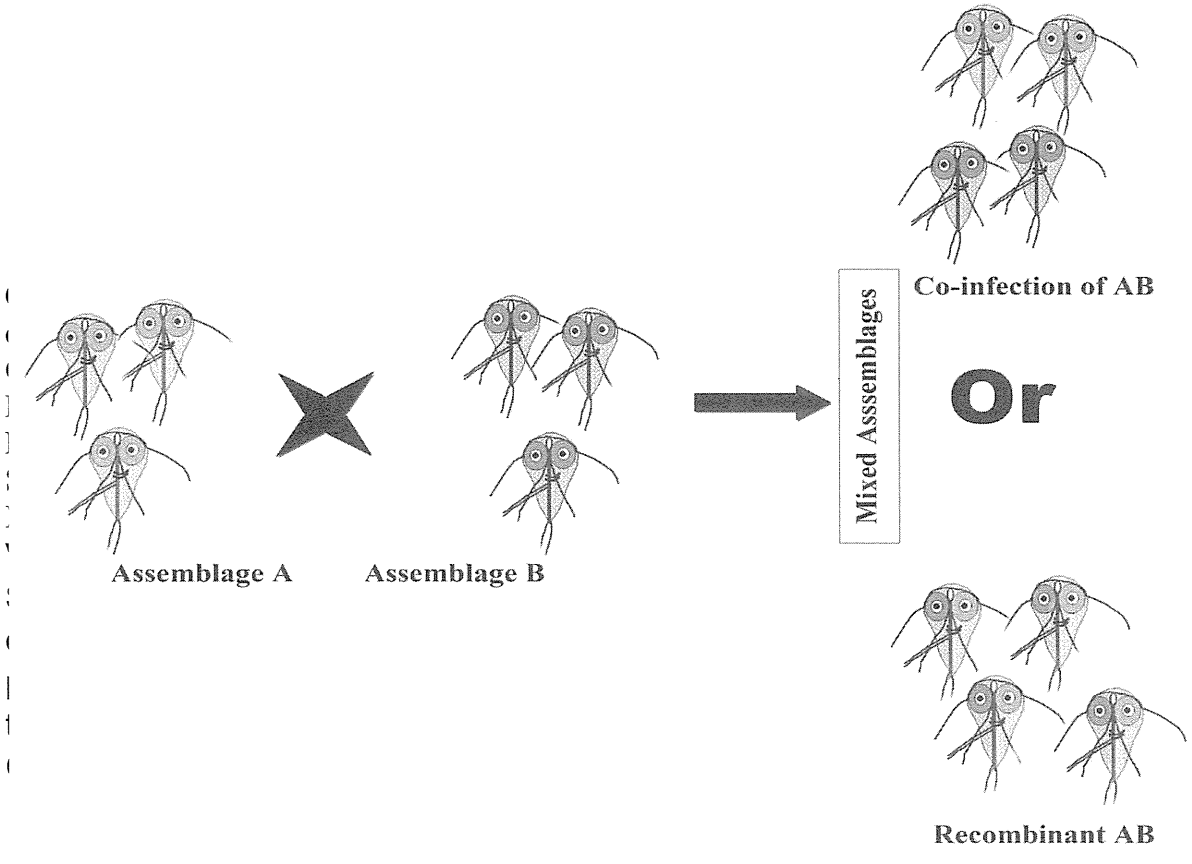
Season	**Average Rain (mm)**	**Monthly average *G. duodenalis* positive cases**	**Total diarrhea cases**	**Monthly average *G. duodenalis* positive (%)**
Pre-Monsoon/ Summer 08	153.4	11	73	15.05
Monsoon 08	1291.7	12.75	103.5	12.02
Post-Monsoon 08	70.3	12	110.3	10.1
Winter 09	3.4	4.5	91	4.8
Pre-Monsoon/ Summer 09	251.8	11.7	123	9.26
Monsoon 09	971.5	18.75	141	13.5
Post-Monsoon 09	95.7	5.7	73.3	7.73
Winter 10	16.6	2	34	6.3
Pre-Monsoon/ Summer 10	143.7	7.3	67	10.83
Monsoon 10	787.4	4	48.25	8.32
Post-Monsoon 10	138.8	4.7	48	10.3
Winter 11	5.4	4	37.5	10.7
Pre-Monsoon/ Summer 11	245.2	5	51.7	10.03
Monsoon 11	1391.6	1.75	35.5	4.87
Post-Monsoon 11	29.5	2.7	32	9.6

Homology analysis showed that the sequences within each assemblage A and B clusters for all the loci are mostly homologous (data not shown) taking all the isolates in account. Also in the combined phylogenetic analysis, these 14 isolates doesn't make any separate cluster and moreover they are evenly distributed in the respective cluster either assemblage A or B similar to Fig. 1. Hence, in this case the outcome of assemblage was solely depended on the marker loci. Association of genotype outcome with other physical factors such as Age, Sex and co-infection status was also checked but no particular association was observed. From this finding it can be said that the differential taxon position of this 14 isolates could not be due to chance and it is a true example of mixed assemblage. Two major reasons can be placed in favor of this outcome i.e. presence of mixed infection or co-infection of two different strain & occurrence of genetic recombination through sexual reproduction. Previous reports of mixed assemblages relied on the presence of multiple peaks in a particular position in the chromatograms which lead to the ambiguous taxon positioning of the isolates in the phylogenetic tree which can be due to mixed infection but, in this case all the isolates with dual genotype are positioned perfectly in a particular cluster for a specific loci. Although, recent reports suggest towards considering this type of observations as mixed assemblage infection, in spite of a thin probability of inter-assemblage recombination, still detailed molecular epidemiological study is required to find out the exact reason behind this unique finding. However, detection of high percentage of mixed genotype is evident, whether it is due to mixed infection or genetic recombination.

Possible hypothesis



- Viral RNA was denatured in the presence of GLV-CF and GLV-CR primers (0.4 mM) by heating at 94 C for 3 min, quick-chilled in wet ice, and subjected to RT-PCR following procedures provided by the manufacturer (Invitrogen).
- [RT-PCR consisted of 1 hr incubation at 53°C, followed by 3 min denaturation at 94°C, and then 40 cycles of 94°C for 30 sec, 53°C for 30 sec, and 68°C for 1 min, followed by a final extension at 68°C for 5 min]
- RT-PCR products were analysed by polyacrylamide gel electrophoresis followed by ethidium bromide staining.

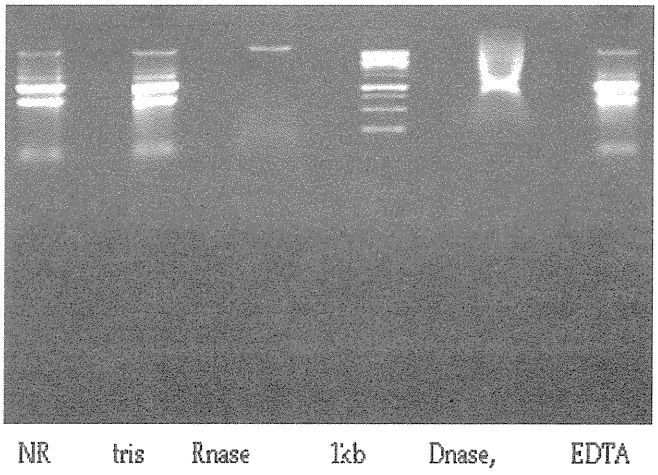


Fig: Isolated RNA with different treatment

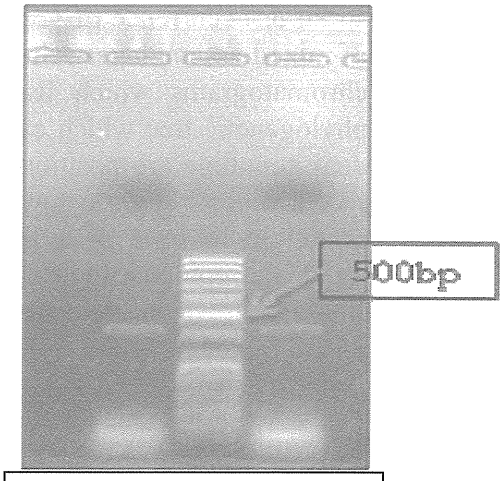


Fig: cDNA and PCR amplification using Superscript III one step RT PCR kit, Invitrogen (using Ref primer for GLV capsid protein.

Sequencing Result: The sequence obtained has no significant identity with reported cds of GLV capsid sequence. (alignment provided as FASTA file named GLV wg_cap_4F)
New PCR primers were designed targeting the conserved region of GLV capsid protein and PCR was done according to the reference protocol.

Name of genes	sequence
Capv1F	5'-CTGGTAATTGCTCACTTTCATC-3'
Capv1R	5'-AACATATCCTTGTAAGCAGACC-3'
Capv2F	5'-GCTCACTTTCATCGTCTATCTT-3'
Capv2R	5'-CGGTGGAAACGTCGAGTG-3'
Capv3F	5'-CTTCGAGAGCTCAATTCCACA-3'
Capv3R	5'-GAAACGTCGAGTGAGGTGG-3'
Capv4F	5'-CATGTCGAATAGAACGAGGTACT-3'
Capv4R	5'-GTAACCATGGAAACATAGGG-3'
Capv5F	5'-GGTCTGCTTACAAGGATATG-3'
Capv5R	5'-AAGAACTGTGGGCCGCTCG-3'

PCR products:

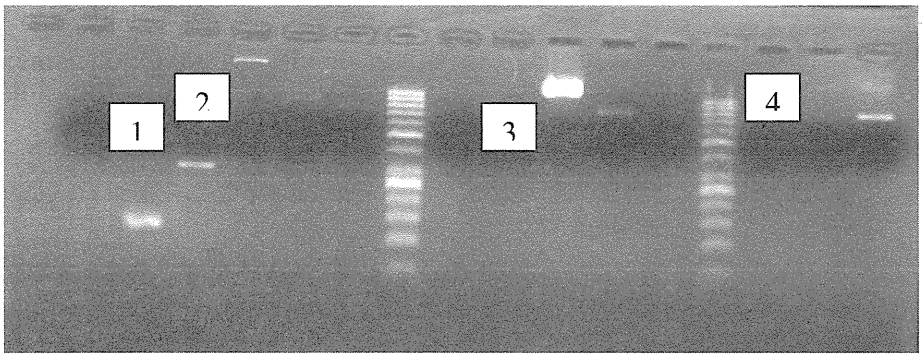


Fig: cDNA and PCR amplification using Superscript III one step RT PCR kit, Invitrogen (using new designed primer for GLV capsid protein.

1 represents: >L21F (primer set 1F/1R)
2 represents: >L31F (primer set
3 represents: >L45R
4 represents: >L51F

Findings:
Although the primers were designed against the GLV capsid protein but most of the PCR products with variety of PCR conditions were non-specific in nature. Few DNA bands from the desired base pair were purified and sequenced with the specific primers but the results obtained were not desirable (Table)

Primer Set	**Identity with (Accession no.)**	**Score**	**E value**	**Query coverage**
L21F	XM_001705748.1	141	9e-31	95%
L31F	XM_001707957.1	326	7e-86	91%
L45R	XM_001706447.1	463	5.5e-16	90%

L51F	XM_001706605.1	582	7e-163	99%
4F	XM_001706802.1	129	0.78	59%

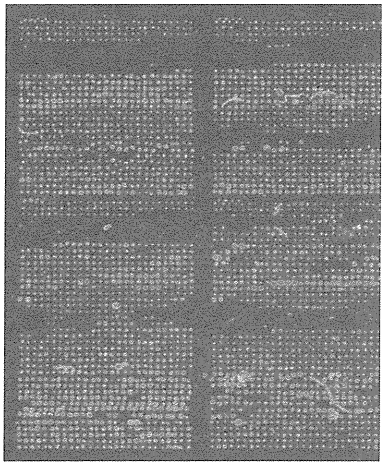
Conclusion:

- The Portland I strain of Giardia lacks GLV
- Local strains of Giardia also lacks GLV
- Thus, differential pathogenesis of Giardia is caused as per host system and genomic or better said transcriptomic and proteomic regulations.

- **Identification of new genes in Giardia inside human GUT (Microarrary hybridization)**

We have used different in vitro procedures for mimicking human GUT, like high oxygen tension etc. to find out what are the differentially regulated factors in Giardia that helps the parasite to live inside the human GUT even at very high oxygen tolerance level than they can withstand. We have used a genomic DNA microarrary for hybridization procedure for fishing out these particulat candidate regulators.

Microarray analysis: The hybridized microarray slides were scanned and more than 200 clones have been identified that show 5 folds or higher times upregulation or downregulation than the control set. The scanned picture (Fig.1) and the analysed result (Fig.2) have been shown below.



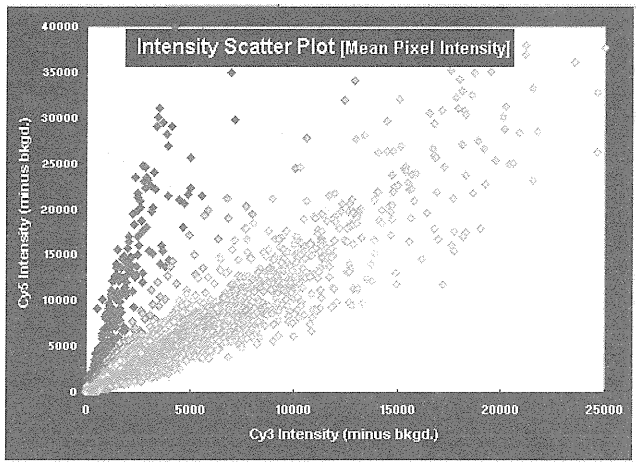
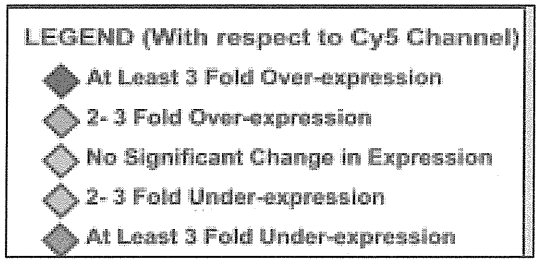


Fig.1 DNA Array hybridization with stressed cell lines : some up-regulated (red) and down regulated (green) spots (genes) are highlighted here.

Fig. 2



a. **Sequencing result:** The result is shown in the following table.

Names of the genes	**Gene_ID**
Metabolic enzymes coding geness	
NADH Oxidase	GL50803_9719
NADH Ferrredoxin Oxidoreductase	GL50803_17151
Pyruvate Ferredoxin Oxidoreductase	GL50803_114609
Thioredoxin Reductase	GL50803_9827
Nitroreductase	GL50803_15307
Arginine deiminase	GL50803_112103
Malate dehydrogenase	GL50803_3331
Alcohol dehydrogenase	GL50803_13350
Phoaphatase and kinase coding genes	
CAM Kinase	GL50803_16034
Serine threonine protein phosphatase	Gl50803_21498
Transcriptional/translational and cell divisional protein coding genes	
Small subunit rRNA	GL50803_r0019
Large subunit rRNA	GL50803_r0013
TAR RNA loop binding protein	GL50803_32741
Nuclear LIM interactor interacting factor-I	GL50803_14905
TMP 55	GL50803_137641

Protein 21.1	GL50803_13590
FtsJ cell division protein	GL50803_16993
Spindle pole protein	GL50803_8512
Structural proteins coding genes	
Beta Giardin	GL50803_4812
Dynein light chain	GL50803_7578
Some other important protein coding genes	
Hsp70B2 cytosolic form	GL50803_88765
Hsp90 alpha	GL50803_98054
Cysteine rich variant specific protein	GL50803_113297
Sodium-hydrogen exchanger III	GL50803_102647
Cathepsin B precursor	GL50803__17516
Hypothetical protein coding genes	
Hypothetical protein	GL50803_17453
,,	GL50803_41258
,,	GL50803_9752
,,	GL50803_11772
,,	GL50803_15039
,,	GL50803_6464
,,	GL50803_16980

,,	GL50803_13274
,,	GL50803_3421
,,	GL50803_113722
,,	GL50803_8509

b. Real time PCR validation: Some of the important genes found from the sequencing result have been checked by Real time PCR. PCR result of Hsp90, nitroreductase and Pyruvate ferredoxin oxidoreductase have been shown below:

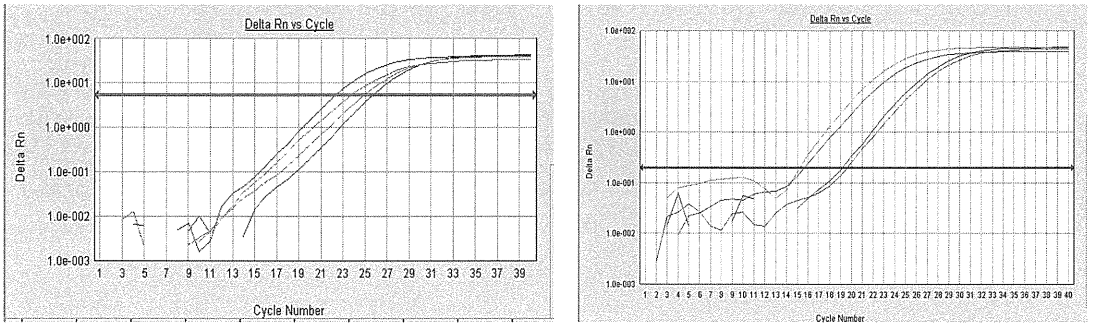


Fig. 3 Differential expression of Hsp90, Nitroreductase (NR) and Pyruvate-ferredoxin oxidoreductase (PFOR) in control and stressed cells

c. 2D analysis: Differential transcription of some genes due to oxidative stress were further analysed in differential protein expression level using 2D gel electrophoresis. The gel picture after silver staining has been shown below:

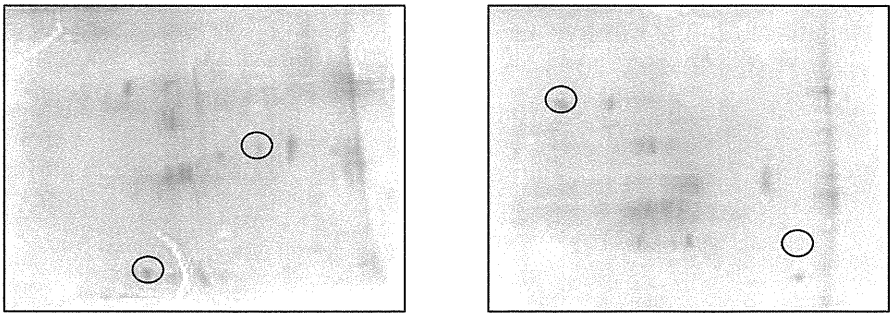


Fig. 5 2D gel electrophoresis. (a) Control set and (b) Oxidative stressed cell set.

vi) Future goals and Plan for fiscal year 2014-2015

1. Further validation of the results obtained in transcriptomic analysis.

2. To find out the regulatory mechanism for these candidate genes in differential pathogenic regulation.

3. To further extend the same in other pathogenic enteric protozoa like *Entamoeba histolytica*.

Publications:

1. Avik K Mukherjee, Punam Chowdhury, Mihir Bhattacharya, Krishnan Rajendran and Sandipan Ganguly. Role of Giardia in diarrhoeal disease regulation in an endemic region. 2014 (Communicated to *BMC Public Health*).

2. Dibyendu Raj, Esha Ghosh, Tomoyoshi Nozaki and Sandipan Ganguly. Differential Gene Expression in *Giardia lamblia* under Oxidative Stress: Significance in Drug Designing and Eukaryotic Evolution. *Gene* 535 (2014) 131–139.

3. Arjun Ghosh, Sumallya Karmakar, Avik K. Mukherjee, Dibyendu Raj, Koushik Das, Srimanti Sarkar, T. Nozaki, & Sandipan Ganguly. THE SPLICEOSOMAL PROTEIN SnRNP F BINDS TO BOTH U3 AND U14 CLASS OF snoRNA IN *Giardia lamblia*. *Global J Bio Agri & Health Sci.* Vol.2(3):178-184. 2013

4. Sumallya Karmakar, Dibyendu Raj and Sandipan Ganguly. Identification of the N-terminal Glycinearginine Rich (GAR) Domain in *Giardia lamblia* Fibrillarin and Evidence of its Essentiality for snoRNA Binding. *Int. J. Trop. Dis. Health* 3(4): 318-327, 2013.

5. Abhishek Sinha; Subhra Ghosh Dastidar; Sandipan Ganguly; Srimonti Sarkar. A unique variation in the evolutionarily conserved Sec61 protein translocon from the protist *Giardia lamblia*. 2013. (Communicated to *PLOS One*).

6. Avik Kumar Mukherjee, Sumallya Karmakar, Dibyendu Raj and Sandipan Ganguly. Multi-locus Genotyping Reveals High Occurrence of Mixed Assemblages in Giardia duodenalis within a Limited Geographical Boundary. *British Microbiology Research Journal*, 3(2): 190-197, 2013

3. *Development of universal Shigella vaccine based on virulence gene expression.*

1. Title of Project

Development of a universal *Shigella* vaccine based on virulence gene expression.

2. Name of investigators

Hemanta Koley[a], JiroMitobe[b], GB Nair[a].

3. Division of Institute where work conducted

a) Division of Bacteriology, National Institute of cholera and Enteric Diseases, Kolkata, India

b) Department of Bacteriology, National Institute of Infectious Diseases, Shinjuku, Tokyo 162-8640, Japan.

4. Summary:

Enteric bacterial infection cause of diarrhea, among them, Shigella species cause of shigellosis as a result infant morbidity and mortality in developed as well as in developing countries. At present, only antibiotic therapy is available for treatment of shigellosis. Unfortunately, due to the global emergence of multidrug resistance, the choice of antimicrobial agents for treating shigellosis is very limited and we are approaching where the shigellosis can become an untreatable disease because of lake of an effective antibiotic. Therefore, the possibilities of other preventive measures such as anti-dysentery vaccines have attracted increasing attention in this field. Various trials of several candidates' vaccine are being done in different parts of the world, but till date no suitable Shigella vaccine is available for public health use. There are different serotypes of Shigella species and their distribution varies between endemic geographical regions. The immune response against Shigella species are serotype-specific, so current immunization strategies have required the administration of live vaccine strains to provide protection against multiple serotypes. In our study, we evaluated the protective efficacy and immune response live attenuated shigella in guineapig model. Constriction and preliminary protection work done by our Japanese scientist in Japan. In India, the protective efficacy after oral immunization with four doses (0, 14th & 28th Day) of Shigella strain was examined. In our protective efficacy studies, we have observed 100% protection (against *S. dysenteriae*) in the immunized group whereas the unimmunized group of animals, thre was noprotection (0%). Serum IgG and IgA showed exponential rise during oral immunization. Vaccine strain MF 4853 showed low reactogenic than wild type strain.

5. **Purpose:**

Current vaccines for bacterial diseases have a serotypic direction that limits the effect of vaccination to a narrow range of bacteria within the same species. An attempt to develop vaccine against broad serotype is worthwhile but difficult. This may result from powerful immunogenicity of serotypic polysaccharide antigen that could camouflage potential antigenicity of common virulence proteins. We have developed a candidate of broad *Shigella* vaccine based on molecular mechanism of virulence gene expression.

Keeping such ideas we started work with the salient objectives are:

a) To understand protective efficacy and immunogenicity of live genetically manipulated shigella vaccine strain against homologous as well as heterologous S*higella* strains in guinea pig model
b) To study the duration of protection offered by live genetically manipulated shigella vaccine strain.

6. Methods Materials:

i) Animals

Two-month-old English colored guinea-pigs of either sex, weighing between 250 and 300 g, were used in this study. Guinea-pigs were collected from the Animal Resource Department, National Institute of Cholera and Enteric Diseases, Kolkata. The study was conducted under dedicated biosafety level 2 conditions with the housing of animals in individually ventilated caging systems maintained at 24 1C with 65% humidity. The guinea-pigs were not starved during the experimental period and no gut sterilization with antibiotics was carried out before the experiment. Before the initiation of the study, the animals were tested *S. flexneri* 2a infections by ELISA against lipopolysaccharides of test pathogens. Institutional animal ethical committee granted approval to conduct this study.

ii) Animal experimental design:

One set of experiment will be conducted by three groups (A, B, C) of male Hartley guinea pig (more than 2 months old).

Protection Study for *S. dysenteriae* Type I (total)

A) Immunization by vaccine strain MF4835--- six guinea pigs +2 for specimen

B) Immunization by wild-type 2457T: Control 1--- six guinea pigs +2 for specimen

C) Naïve, administration of saline : control 2--- six guinea pigs +2 for specimen

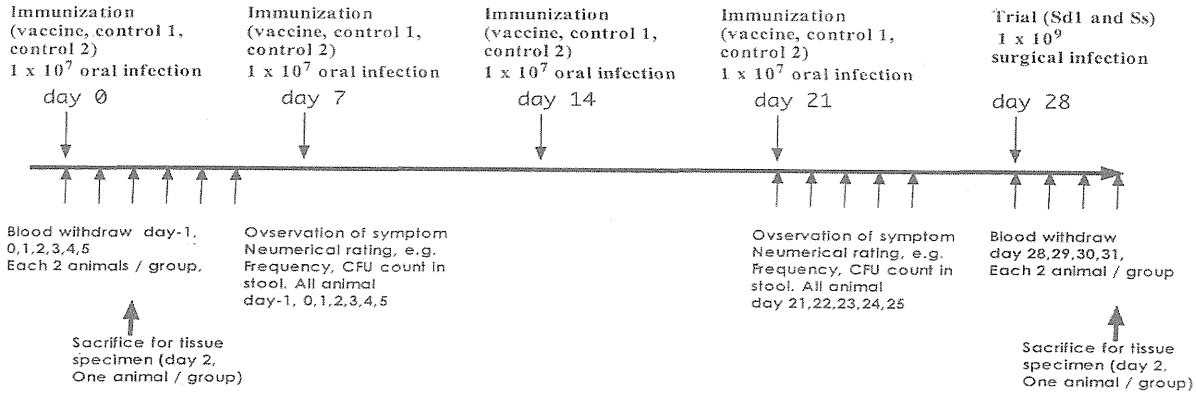
iii) Immunogen preparation and immunization

The overnight growth of *Shigella flexnari* 2a 2457T and Vaccine strain *Shigella flexanari* MF 4853 was scrapped off from TSA and suspended in PBS and centrifuged (10 min, 10 000 g). The resulting pellet was washed twice and resuspended in PBS. The bacterial suspension was adjusted to an OD 600 nm of 1.5. The suspension was adjusted again to OD 600nm 1.5 and was stored at 80^0C till use for oral immunization. OD 1.5 corresponded to 10^7 CFU/ml. Guinea-pigs were anesthetized using a mixture of ketamine (35 mg kg_1 of body weight) and xylazine (5 mg kg_1 of body weight). Guineapigs were orally immunized with 10^7 CFU of **Shigellaflexnari 2a 2457T and Vaccine strain *Shigella flexanari* MF 4853** strains in lmL of PBS under anesthesia. Control guinea-pigs were treated with sterile PBS. The immunization schedule was followed on the 0, 7th, 14th and 21st day.

iv) Flow chart for oral immunization:

Step	Time	Treatment
I	-36 hours	Experimental Guinea pigs starved but given water *at libitum*
II	-18 hours	Intravenous injection of Ranitidine (2mg/kg body weight)
III	-35 minutes	Anaesthetized by Ketamine (35mg/ml) and Xylazine (5mg/kg body weight, intramuscular)
IV	-15 minutes	Neutralization of hydrochloric acid by 5%, 5 ml Sodium bicarbonate
V	0 minute	Again administered 5 ml of 5% Sodium bicarbonate along with 1ml (10^7) WILD TYPE STRAIN *Shigella flexenari* 2a 2457T and VACCINE STRAIN MF 4831.

v) Animal experimental Schedule



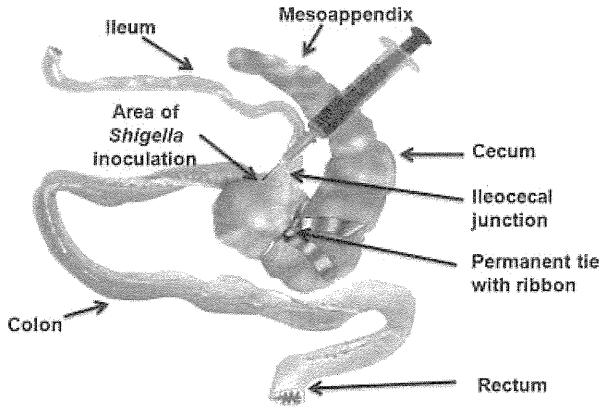
vi) Surgical procedure:

The test animal was sedated by an intramuscular injection of a mixture of ketamine (35 mg kg_1 body weight, Sterfil Laboratories Pvt Ltd, India) and xylazine (5 mg kg_1 body weight, AstraZeneca Pharma India Ltd, India). The cecum was brought out through a 3 cm midline incision without compromising the blood supply. A

permanent cecal tie was made 4 cm apart from the ileocecal junction so that the ligation completely obstructed the cecal lumen above this junction while maintaining the ileo–ceco–colic connection. The purpose of this ligation was to prevent the entry of cecal contents into the proximal colon and disruption of water absorption. During the surgery, hydration of the exposed intestine was maintained with sterile PBS. At the cecocolic junction, 1mL of test inoculum was injected into the lumen of the colon. The colon was placed back inside the abdominal cavity and the incision was closed. The incision site was checked twice a day for signs of infection, and each time, it was washed with a 1% chlorhexidine solution soaked with sterile gauze pads during the next 72 h. We did not find any wound infection in any of the guinea-pigs during the postsurgical period. After the surgery, the animals were allowed to consume food and water and were observed for the development of shigellosis for 48 h. Luminal inoculation with guinea-pigs without cecal bypass was also carried out with 2457T to assess the effects of cecal bypass on the development of shigellosis.

vii) Challenge Efficacy study:

Step	Time	Treatment
I	-36 hours	Experimental guinea pig was starved but water will be given *at libitum*
II	-15 minutes	Guinea pig will be anaesthetized through intramuscularly by ketamine (35 mg/kg) and xylazine (5 mg/kg body weight)
III	- 5minutes	Colon was brought out through a midline incision aseptically
IV	-3 minutes	Permanent tie was placed 2 cm apart from ileocecal junction
V	0 minute	1 ml of bacteria was injected to the lumen ileocecal junction of colon
VI	5 minutes	Animals were allowed to take food and water and observed for the development of shigellosis for 48 hours



Surgical sketch of guinea-pig colon for the experimental shigellosis model

viii) Monitoring of challenged animal:

The challenged animals were monitored for 48 hr after the rectal challenge. They were observed twice daily, every morning and evening, for general activity level, tenesmus, consistency of stools passed into the drop pan of their cages and the amount of blood or mucus observed in the feces (if any). Body weight and rectal body temperature were measured.

ix) Collection of stool sample and quantification of bacteria (shedding)

Stool samples of animals of both immunized and control group were collected from the drop pan. The samples were suspended to make 1 g stool/mL of PBS, 10-fold serially diluted and plated on Hekton enteric agar (HEA, Difco) and MacConkey agar plates (Difco) for bacterial counting. Representative colonies were subjected for the confirmation with appropriate typing sera (Denka Seiken, Tokyo, Japan).

x) Collection of intestinal tissue and nature of colonization of bacteria

After laparotomy, appropriate length (3–4 cm) of the intestine (distal colon) was excised. The excised intestinal tissues were minced, mixed with 3 mL PBS and homogenized with a pestle (Himedia, Delhi, India). The homogenized tissue was added with PBS up to 5 mL in volume. After 10-fold serial dilution of the sample, bacterial count was made on HEA (Difco) and MacConkey agar (Difco). Representative colonies were subjected for the confirmation with appropriate typing sera (Denka Seiken, Tokyo, Japan).

xi) Collection of blood sample

Blood samples of both the immunized and control groups were collected from the foot vein on days 0, 7, 14, 21, 28, 35, 42 and 63. Number of samples collected were 17 from each of the immunized and control group on days 0, 7, 14, 21 and 28, and were seven from each group on days 35, 42 and 63, since 10 each were challenged on day 28 and killed thereafter. Collected blood was allowed to clot at room temperature for 30 min and then kept at 4◦C for 24 hr. Serum was separated from the clotted blood by using a sterile Pasteur pipette and centrifuged at 1000 *g* for 10 min. The supernatant was collected and stored at –20◦C until use.

xii) Immunological Assay :

Analysis of immunogenicity of vaccine strain were performed using ELISA, essentially following the method developed by Keren (27). Disposable polystyrene (Nunc,Denmark) microtiter wells were coated with 10^9 cells/ mL of vaccine strain and incubated for 18 h at 4 °C. Wells were washed three times with PBS (pH 7.4). Control wells were coated with 100 μL of PBS. After the plate was incubated at 4◦C for 18 hr, wells were washed three times with PBS with 0.5% Tween 20 (PBS-T). Non-specific binding sites were blocked by incubating thewellswith 200μLof 5%non-fat dry milk (Bio-Rad, Hercules, CA, USA) at 37◦C for 2 hr. The wells were washed thrice with PBS-T and incubated with serially diluted samples at 37◦Cfor 1 hr. After washing with PBS-T, 100 μL of horseradish peroxidase-

conjugated goat anti-guinea pig IgG (Sigma, St. Louis, MO, USA) or horseradish peroxidase-conjugated sheep anti-guinea pig IgA (ICL, Portland, OR, USA) diluted 1:2000 times in PBS were added to each well and the plate was incubated at 37◦C for one hour. Following washing with PBS-T, 100 μL of OPD (1 mg/mL) dissolved in 100mMcitrate buffer (pH 4.5) containing 0.2% hydrogen peroxide were added to each well. The reaction was stopped after 10 min by adding 100 μL of 2 N sulfuric acid and the resulting color was read at 492 nm using an ELISA reader (Bio-Rad). The readings of PBS control wells were subtracted from those of the corresponding test wells to yield the net optical density. The endpoint ELISA titer was the highest reciprocal dilution yielding a net optical density of 0.100 or greater.

xiii) Histology of intestinal tissue:

After laparotomy, the segment of the intestine from the distal colon to the rectum was sectioned, opened and inspected for signs of mucosal edema, exudation, hemorrhage, ulceration, necrosis and perforation. Samples were fixed in 10% neutral buffered formalin, dehydrated with alcohol and embedded in paraffin. The samples were cut 3 μm in thickness and stained with hematoxylin and eosin

7) Result:

i) Bacterial Shedding of immunizing strain from stool of animals:

Bacterial Shedding of the immunizing strains, wild *Shigella flexnari* 2a (2457T) and vaccine strain MF4853 were measured from stools of immunized animals. After each immunization time (day 0- 7), stools were collected for three consecutive days to identify shedding of both strains. Irrespective of the time of immunization, a quite significant number of the immunizing strain was recovered from stools collected at 24 hr after the immunization. However, at 48 hr after the immunization, the number of the immunizing strain recovered

decreased tremendously and no immunizing strain was recovered at 96 hr after the immunization.

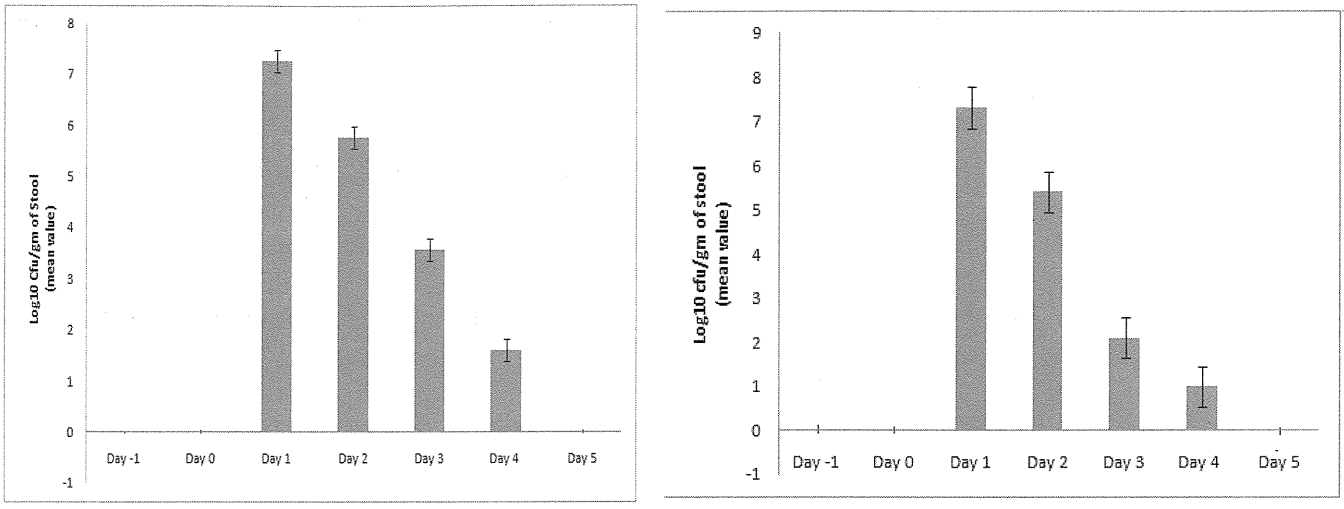


Fig 1: Nature Of Bacterial Shedding of Wild type *Strain Shigella flexnari*2a (2457T) and Vaccine Strain (MF 4853) After 1st Oral Immunization.

ii) Protective efficacy in guinea-pigs

Table 1 shows protective efficacy of immunization of vaccine strain MF4853 against challenge with *S. dysenteriae* 1 NT4907. In the experiment, a total number of 6 animals of the immunized group were orally administered 10^8 cfu of MF4853 in 1mL of PBS. Also 6 animals of the control group were administered 1mL of PBS .On day 28 after the initiation of the immunization (7 days after the last administration) 20 animals of each immunized and control group were challenged with 10^9 cfu of *S. dysenteriae* 1 NT4907 in1mL of PBS.No dysentery symptoms were observed in any animals of the immunized group, which were challenged on day 28 (6 animals). Only one animals challenged on day 28 excreted semisolid stools within 24 hr after the challenge but recovered spontaneously within 48 hr. On the other hand, all animals of the control group challenged on day 28 developed symptoms of bacillary dysentery, such as tenesmus and mucoidal and bloody diarrhea. In all animals, tenesmus characterized by a sudden cramp that caused the body to rise up for an instant, frequently

began to occur at around 24 hr after the challenge and was ob servable during the subsequent 24 hr. Mucoidal bloody diarrhea and mucoidal diarrhea without blood

were observed within 24 hr after the challenge in some animals challenged on day 28. In the control group,

elevation of the rectal temperature by approximately 1.6◦C at 24 hr after the challenge on both day 28 and loss of body weight (approximately 11%) within 3 days after the challenge on both day 28 a were observed. No such observation was made in the immunized group.

Table No.1 Protection Effacacy After Challenge With S. dysentriae type 1 NT4907

Experimental animal	Immunogen used	Challenged Strain used in intestine	Number of animal used	Disease symptoms	% of death with Shigellosis	% of protection against Shigellosis
PBS Control Group	PBS	Wild type *S. dysentriae*type 1*(NT4907)*	6	Shigellosis 100% (6/6)	100% (6/6)	0% (0/6)
Immunized Group	VACCINE STRAIN MF4831	Wild type *S. dysentriae 1(NT4907)*	6	16.3% (1/6)	0 % (0/6)	100%
	Wild Type Strain (S. flexneri 2a 2457T)	Wild type *S. dysentriae* 1*(NT4907)*	6	32% (2/6)	0 % (0/6)	100%

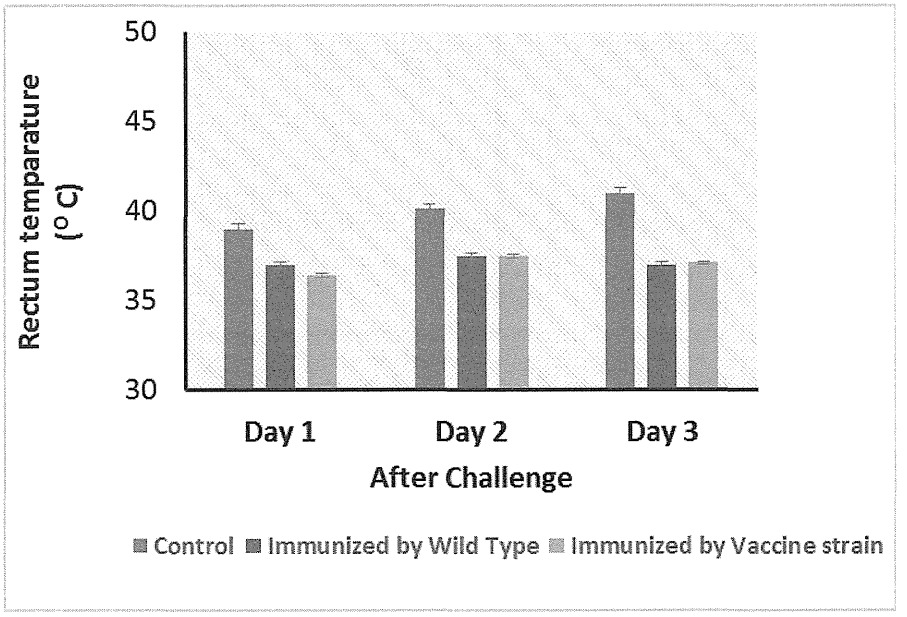


Fig 2: Rectum temperature of Immunized and Non immunized Guinea pigs After Challenge by *Shigella dysentriae* 1.

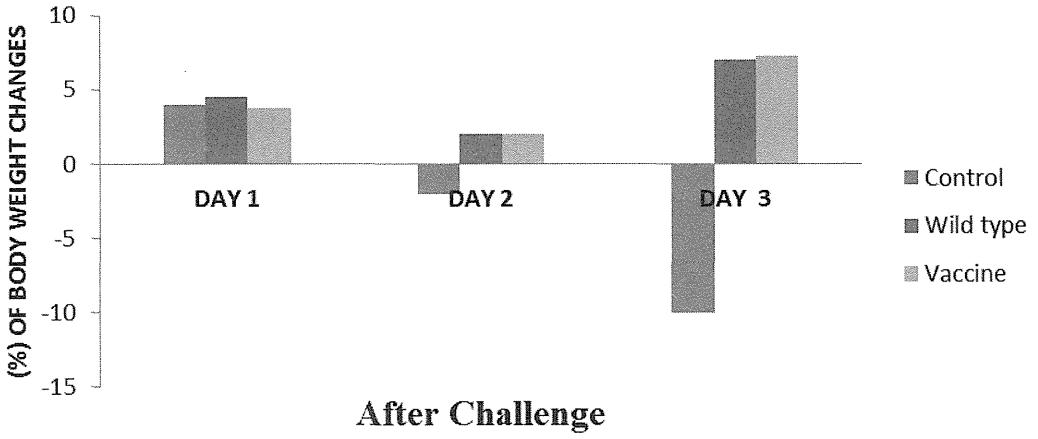


Fig 3: Graphical Representation of Body weight of Animals after Challenge by *Shigella dysentriae* 1.

iii) Recovery of challenged strain from distal colon of animal:

Figure4 shows the results of recovery of challenged *S. dysenteriae* 1 NT4907 from the distal colon of animals of both the immunized and control groups. In animals challenged on day 28 after the initiation of the immunization, at 24 hr after the challenge, at 100± 2×10^2cfu per gram of tissue were recovered from animals of the immunized group, whereas 1.5±2.8 $\times10^9$ cfu per gram of tissue were recovered from animals of the control group.