Immunization with recombinant fusion protein rVE induces CD4+ and CD8+ T-cell mediated memory immune protection against *Yersinia enterocolitica* O:8 infection in mouse model

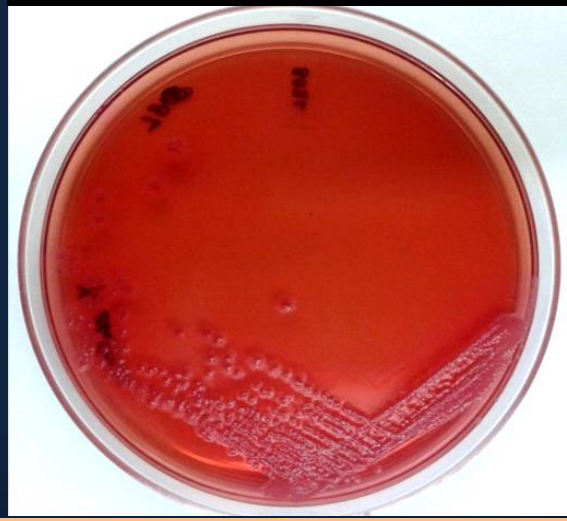
Presenting By
Amit Kumar Singh
Ph.D. Scholar

Supervisor/ Guide
Dr. Joseph J. Kingston, Sc D
Head of Department,
Microbiology Division,
DFRL, DRDO, Mysore

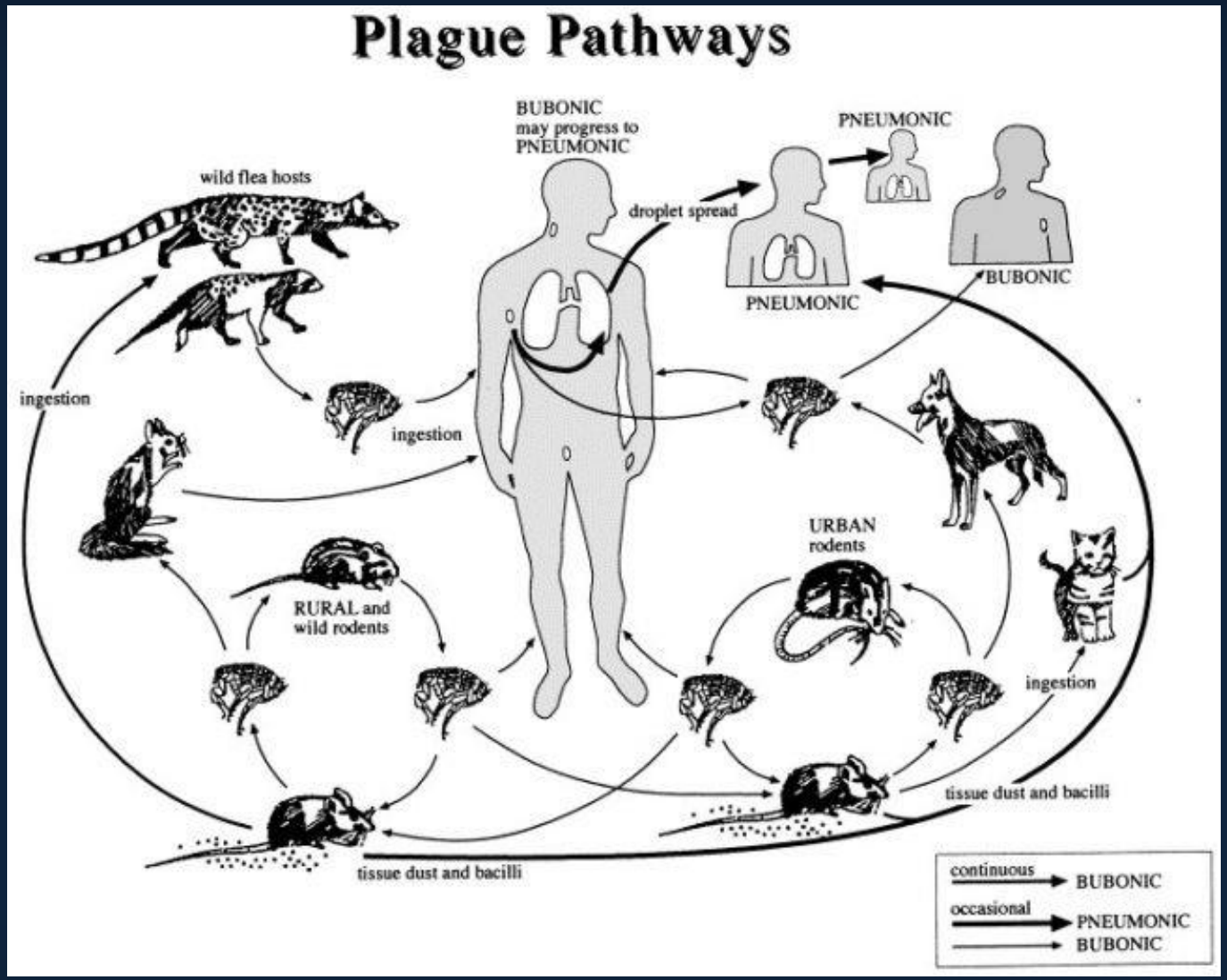
Introduction

Yesinia pestis

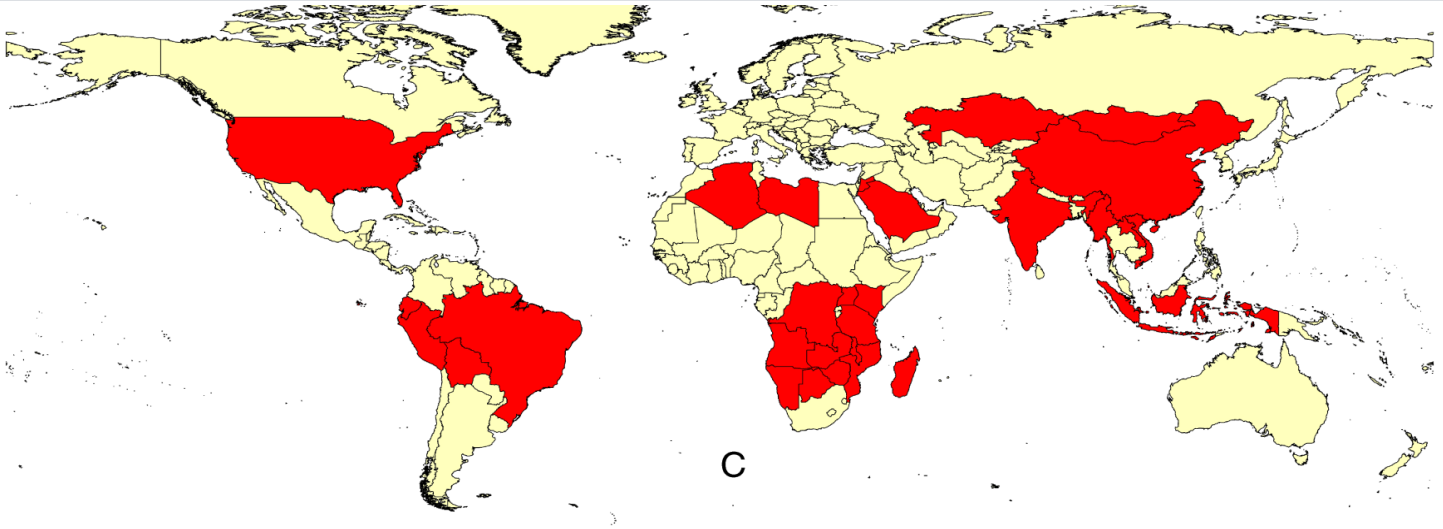
- Gram –ve, facultative anaerobic bacteria.
- Discovered by Alexandre Yersin (1894).
- Family- Enterobacteriaceae.
- Grows at temperatures of 4°C - 40°C (optimum 26°C).
- Zoonotic disease occurs with the bite of infected flea.
- Disease caused:
    1. Bubonic plague
    2. Septicemic plague
    3. Pneumonic Plague

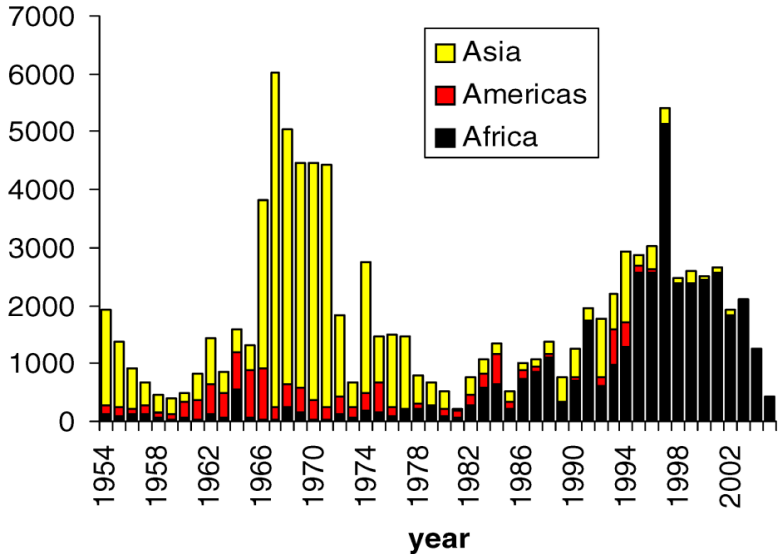


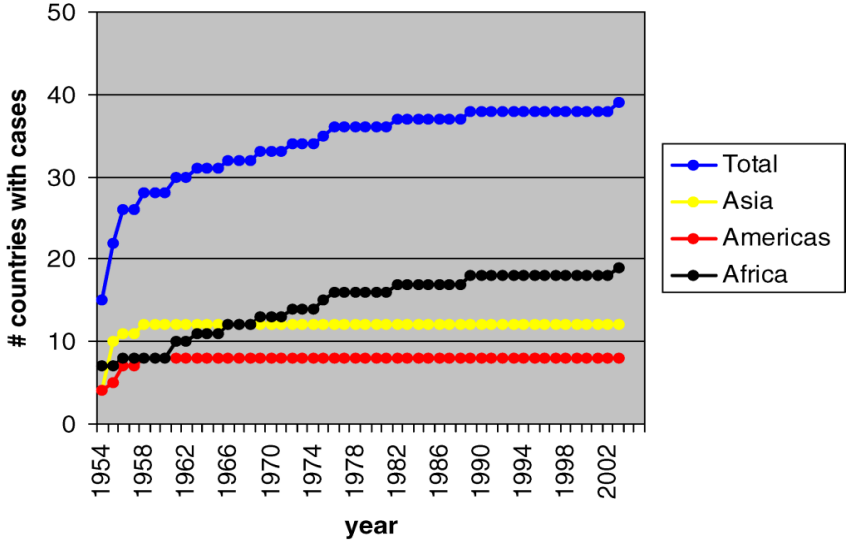
Introduction



Introduction



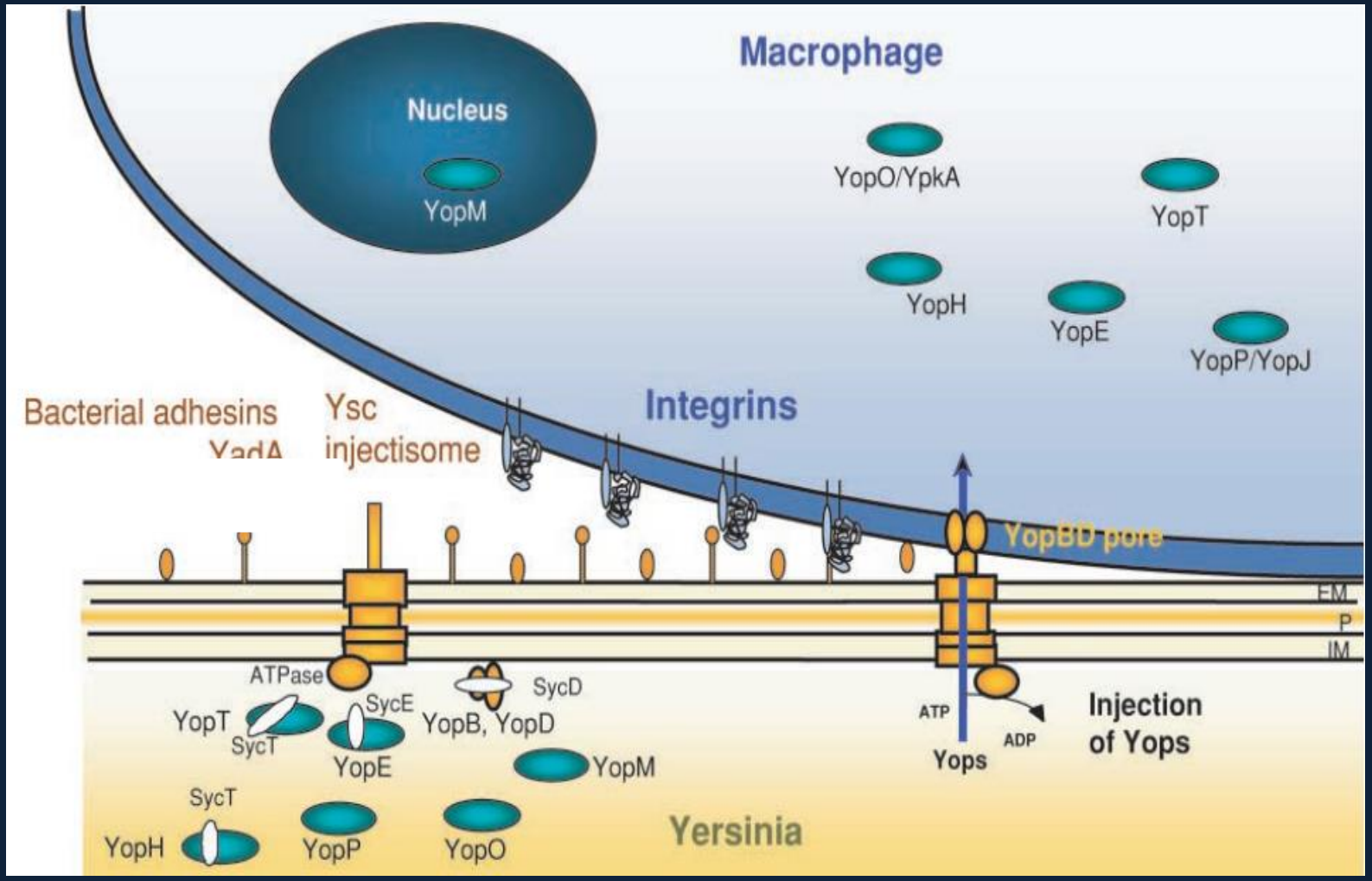




Global occurrence of plague outbreaks

Introduction

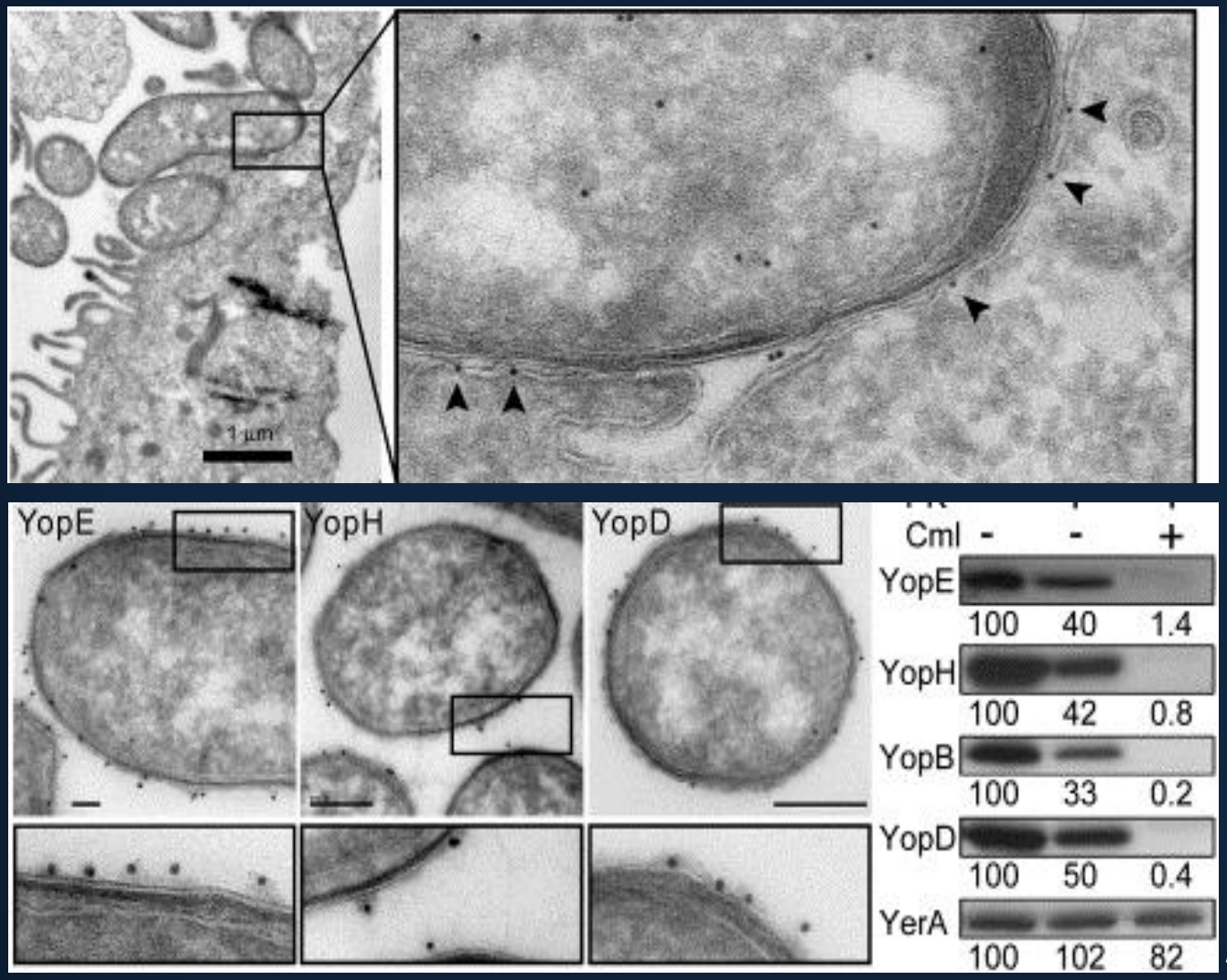
LcrV dependent translocation of effectors in *Yersinia* Type III Secretion system



Cornalis *et. al,.* 2002

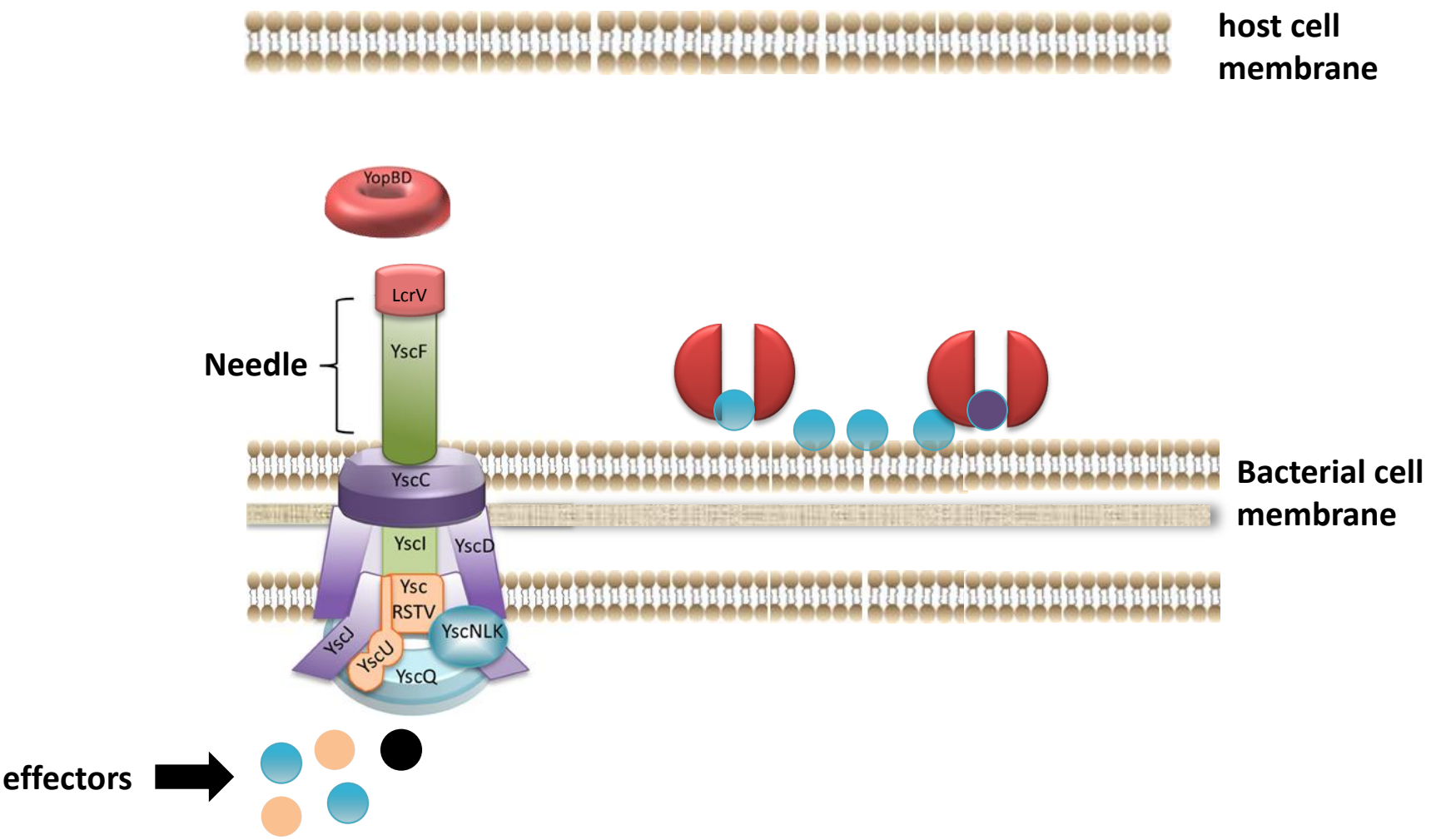
Introduction

LcrV independent translocation of effectors



Akopyan *et. al.*, 2011

Introduction

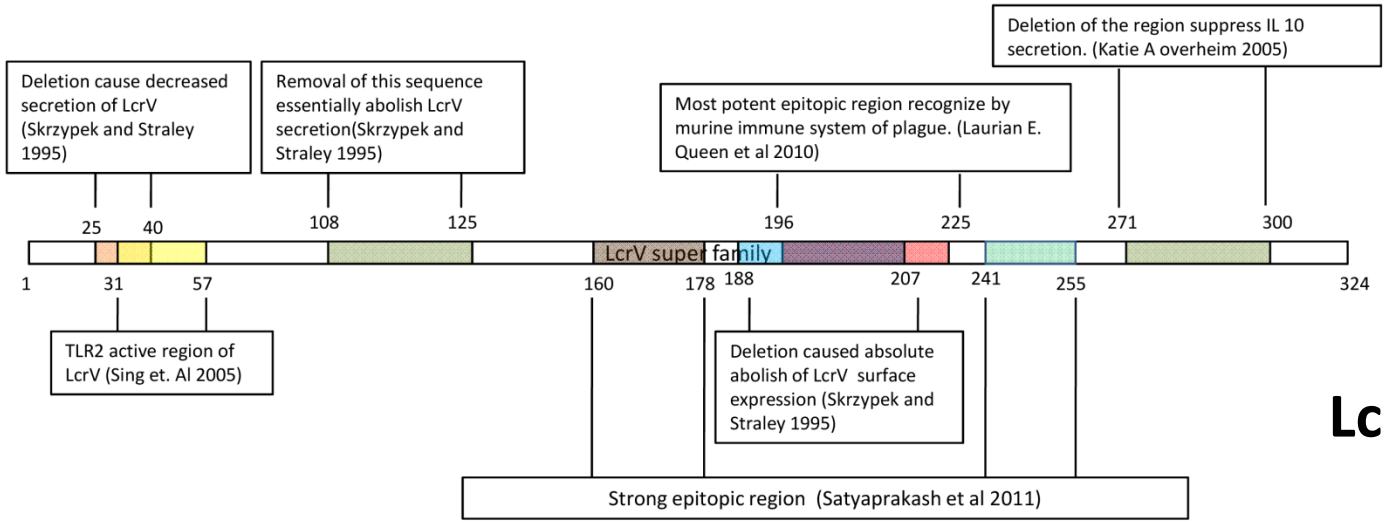


Introduction

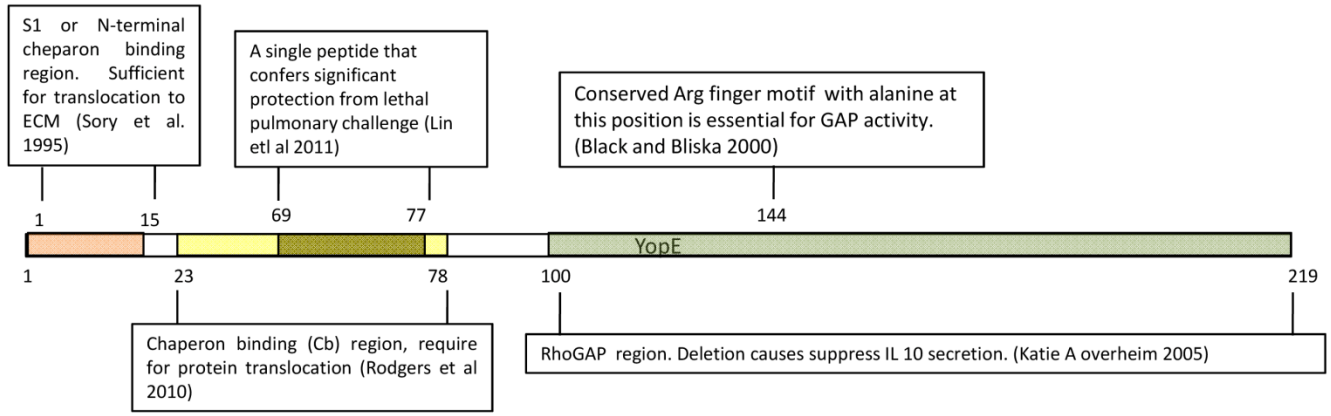
S. No.	Molecule	Mol. wt.	Suspected Function
1	YadA	45kDa	Surface adhesion molecule, helps in attachment with host cell membrane.
2	YopC	49.6kDa	Form outer membrane ring of TTSS.
3	YopD	27.36kDa	Form inner membrane ring together with YscJ. Along with YopB it help in pore formation.
4	YopE	29 kDa	Molecule recognize most by CD8 immune response upon infection. Destabilizing cell cytoskeleton by degrading actin microfilament.
5	YscF	9kDa	~ 150 copies of YscF assemble to form hollow needle.
6	YopH	51kDa	Most phosphorylated effector molecule. Initiate apoptotic like changes in host cell. Prevent cell from being phagocytosis by inhibiting immune response.
7	YopM	51.7kDa	Function remains unknown
8	YopN	32kDa	Prevent premature secretion of effector molecules
9	YopP/ YopJ	32.2kDa	Effector molecule which control length of injectisome. Inside host cell it trigger apoptotic reaction.
10	LcrV	37 kDa	Forming the tip of needle in TTSS. Help in docking on the host cell membrane
11	Syc	29kDa/ dimer	Molecular chaperon, help in secretion of effector molecules. Binds with specific molecules and maintain them into partial unfolded condition

Introduction

Immuno-dominant region of LcrV and YopE

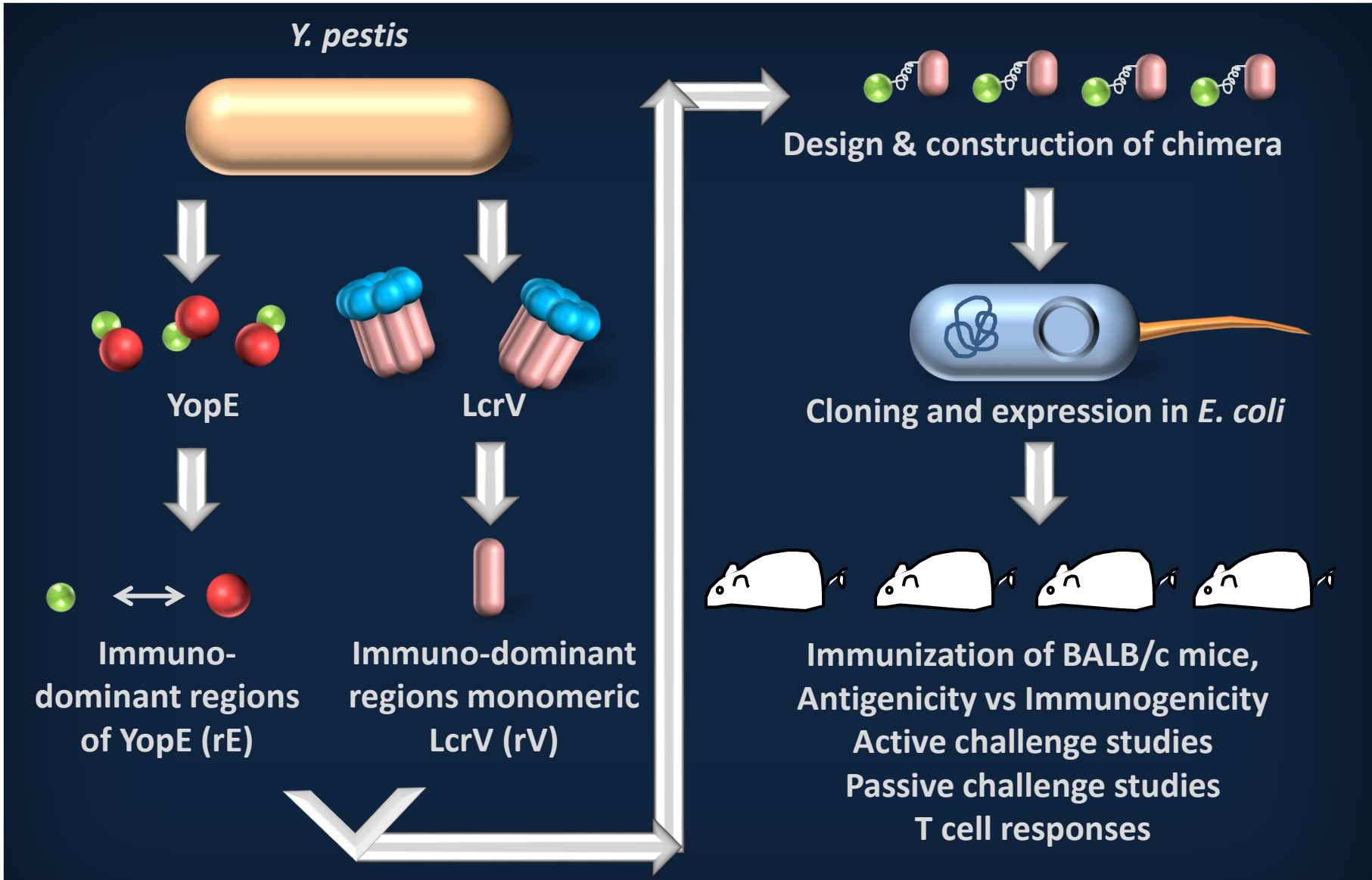


LcrV



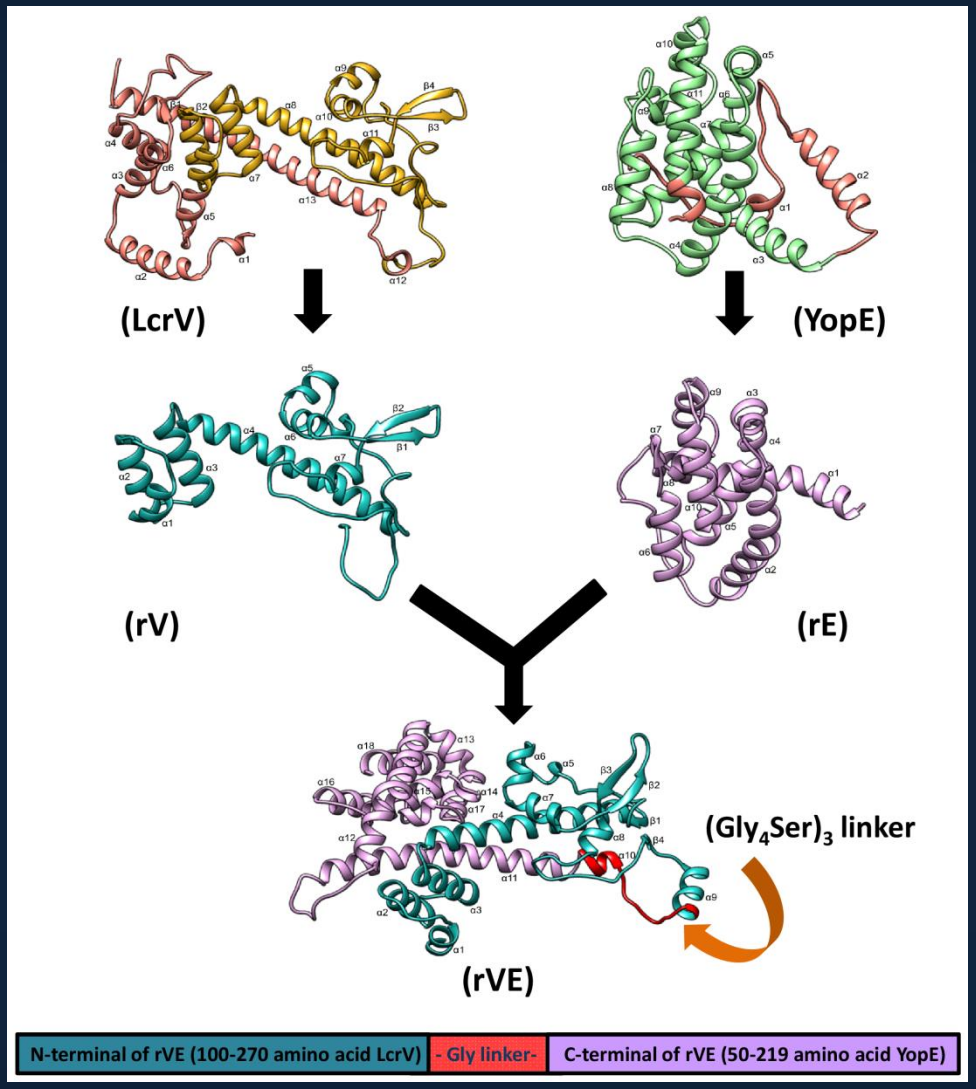
YopE

Plan of work


Y. pestis
YopE
LcrV
Immuno-dominant regions of YopE (rE)
Immuno-dominant regions monomeric LcrV (rV)
Design & construction of chimera
Cloning and expression in E. coli
Immunization of BALB/c mice,
Antigenicity vs Immunogenicity
Active challenge studies
Passive challenge studies
T cell responses

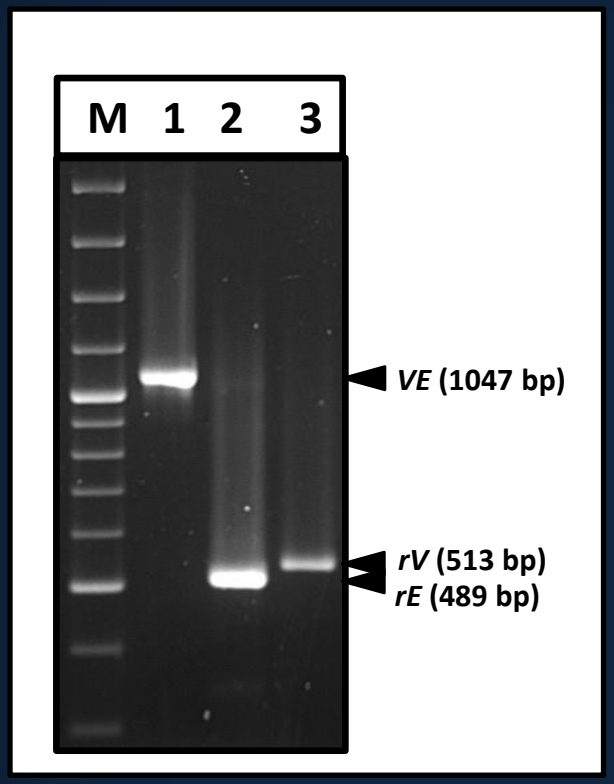
Results

***In-silico* structure prediction of *Y. pestis* LcrV and YopE**

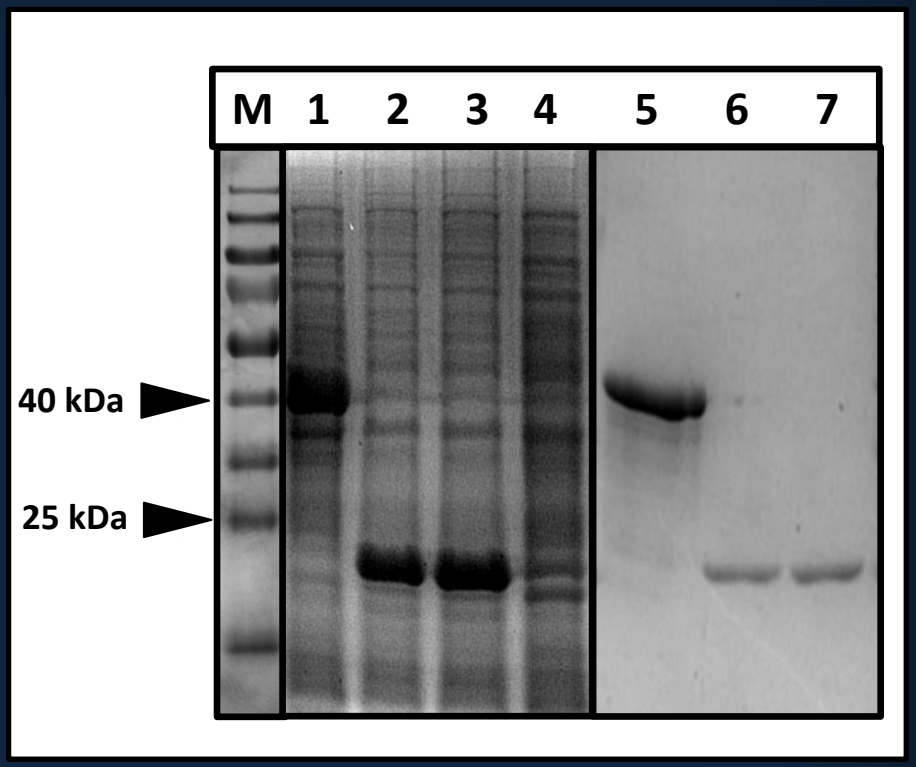


Results

Construction, Cloning, expression and purification of rV, rE and rVE



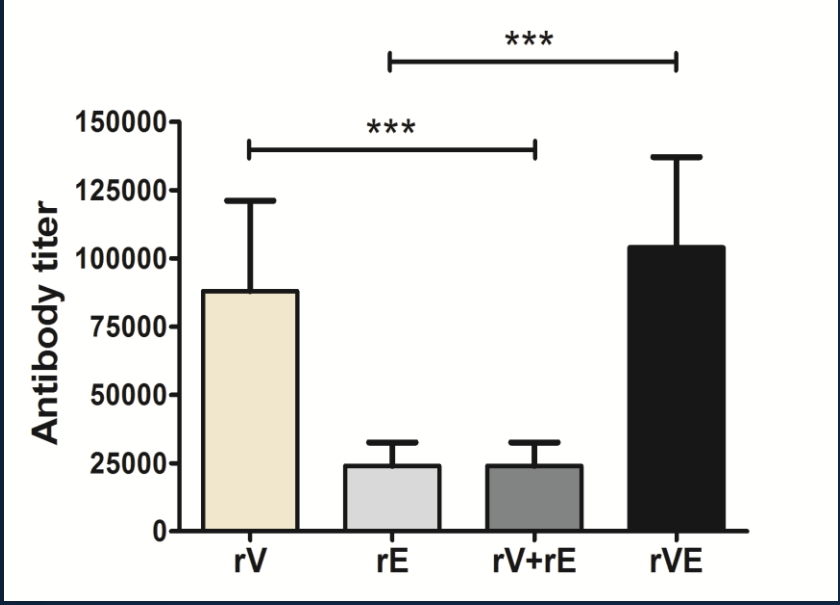
Construction of chimeric gene *rVE*



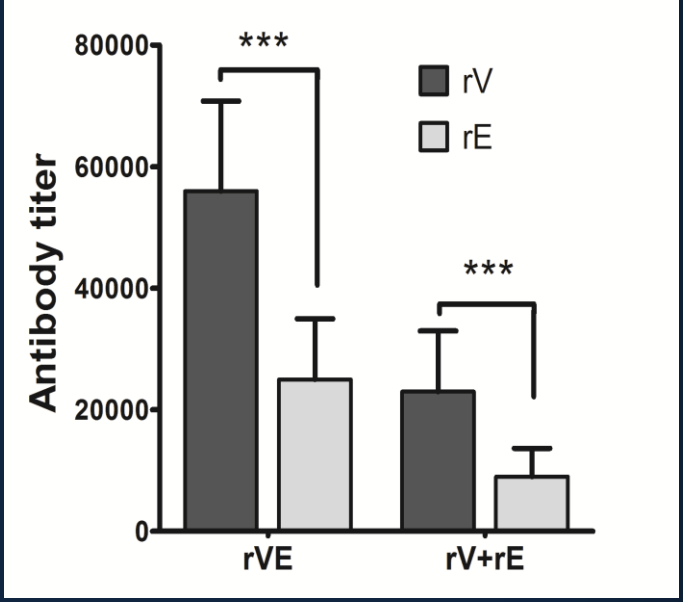
M: Protein ladder 1: Induced rVE clone
2: Induced rE clone 3: Induced rV clone
4: Un-induced rVE 5: Purified rVE protein
6: Purified rE protein 7: Purified rV protein

Results

Humoral Immune responses



Antibody titer after 42nd day immunization

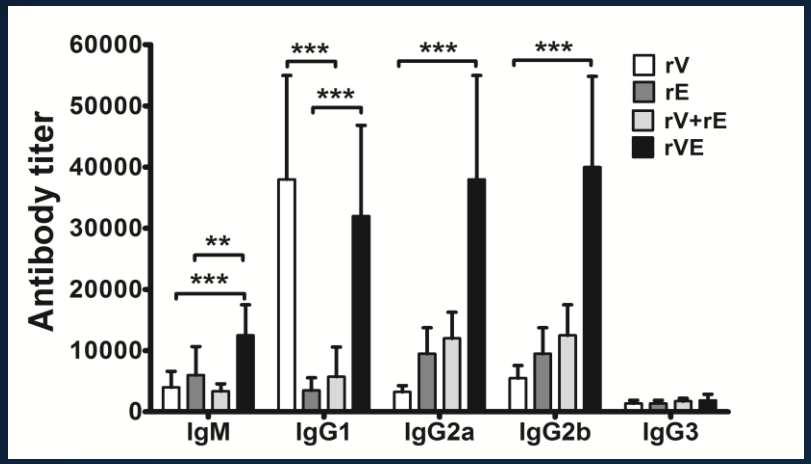


Immuno-competitive assay

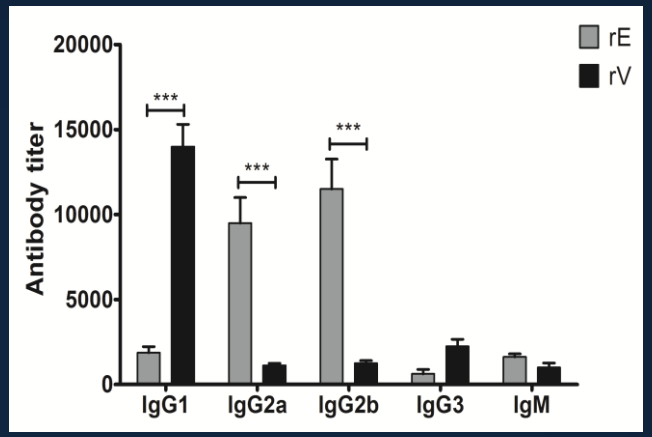
Results

Antibody Isotype profile

(A)



(B)

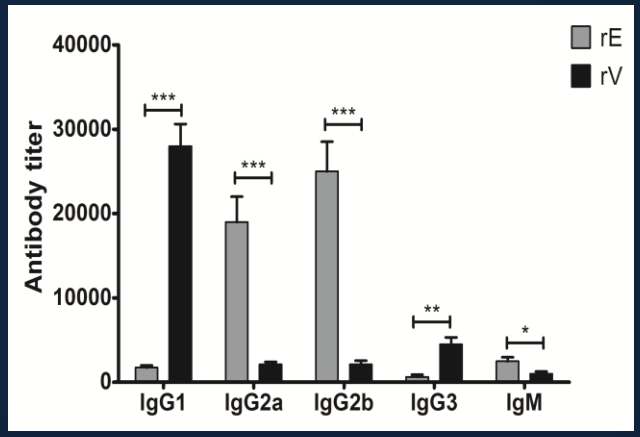


(A) Antibody isotypes induced by individual recombinant proteins (rV, rE), their cocktail mixture (rV+rE) and fusion construct (rVE) in BALB/c mice.

(B) Immuno-competitive assay with anti-rV+rE sera.

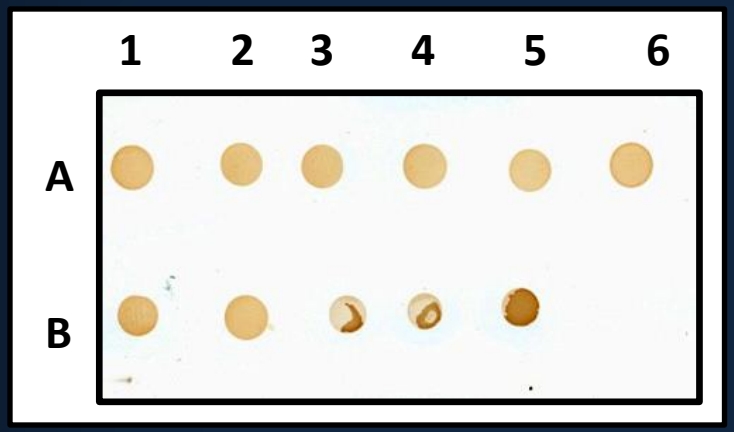
(C) Immuno-competitive assay with anti-rVE sera.

(C)

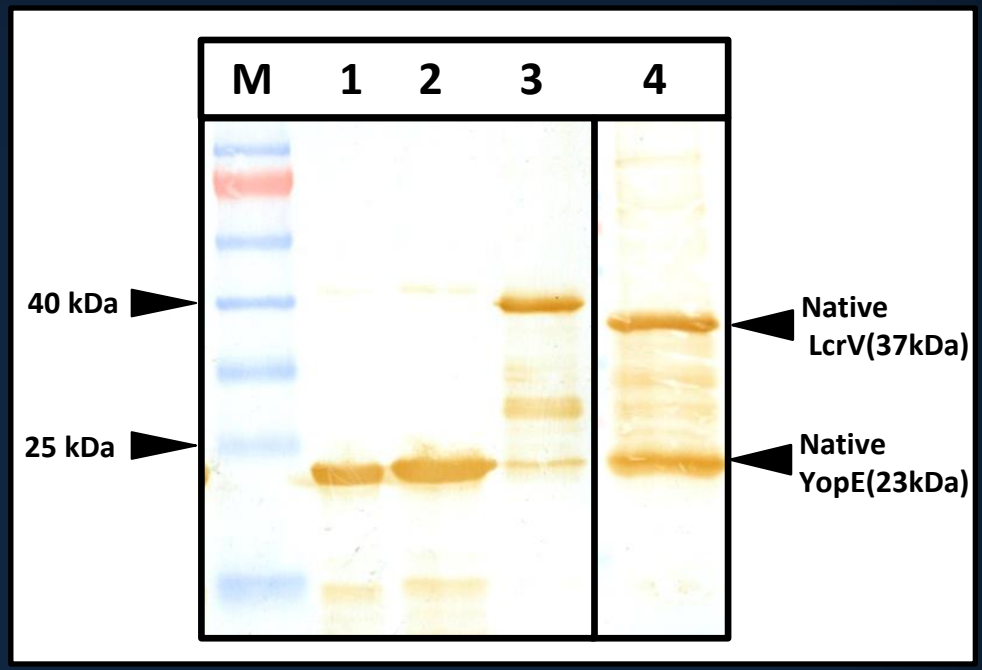


Results

Dot Immunoblot and Western blot analysis



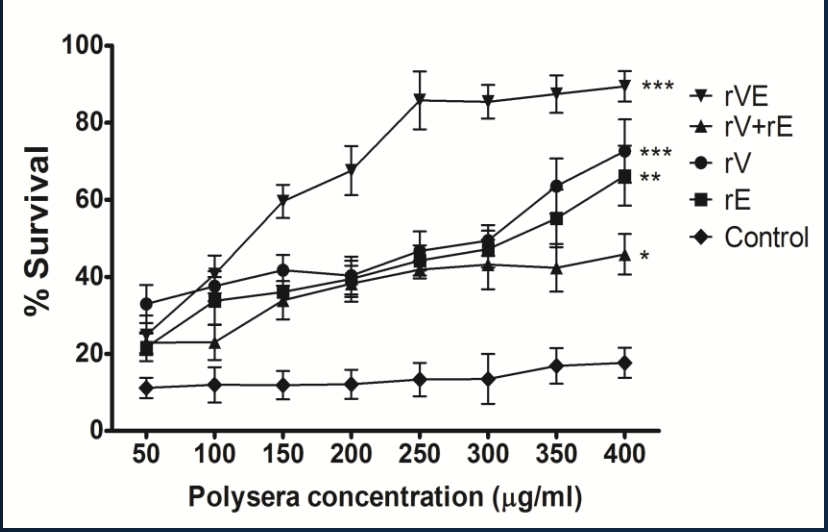
Dot immunoassay analysis to assess binding efficacy of poly-sera raised against VE fusion protein. Lane1 A1-A6: *Y. enterocolitica* O:3, O:5, O:8, O:9 O:13 and O:21 respectively. Lane B1-B6: *Y. enterocolitica* ATCC 23715, *Y. pseudotuberculosis*, rV, rE, rVE and –ve, respectively.



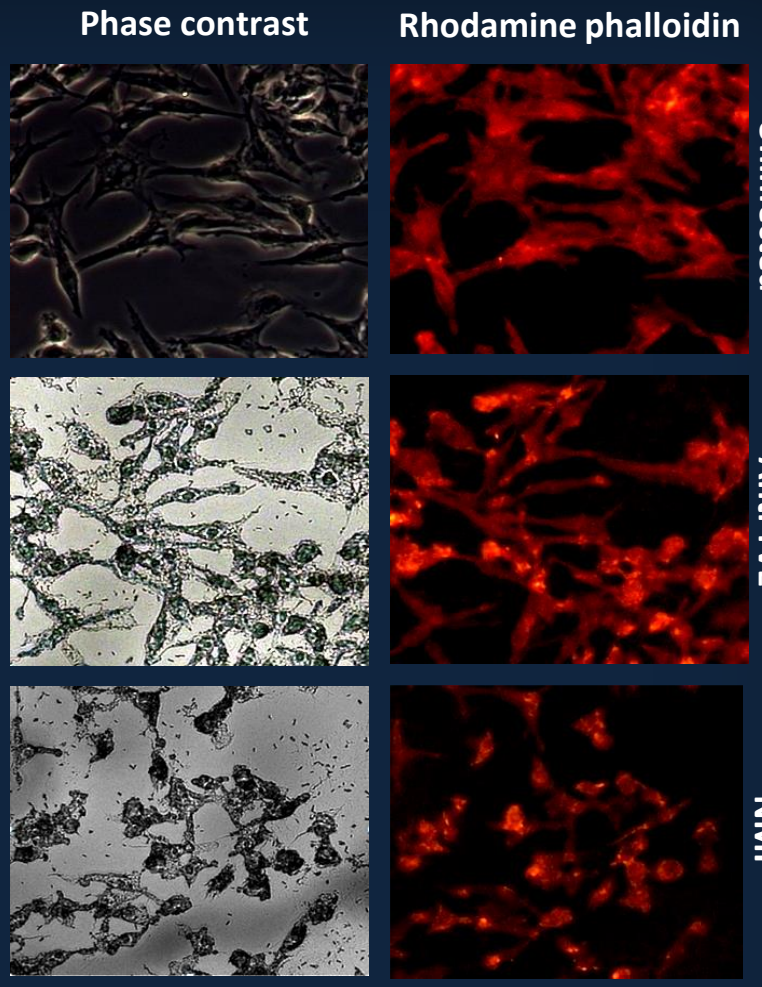
Western blot analysis using anti-*VE* polysera (1:1000) dilution. Lane M: Prestained Protein marker, Lane 1, 2 and 3: whole cell lysate of *E. coli* BL21DE3 recombinant rV, rE and rVE clones. Lane 4: *Y. enterocolitica* O:8 whole cell lysate showing binding efficacy of anti-rVE polysera with native LcrV and YopE proteins.

Results

In-vitro assessment of protective efficacy



Protection of RAW 264.7 cell line from *Y. enterocolitica* O:8 induced cytotoxicity by anti-rV, anti-rE, anti-rV+rE and anti-rVE polysera raised in BALB/c mice

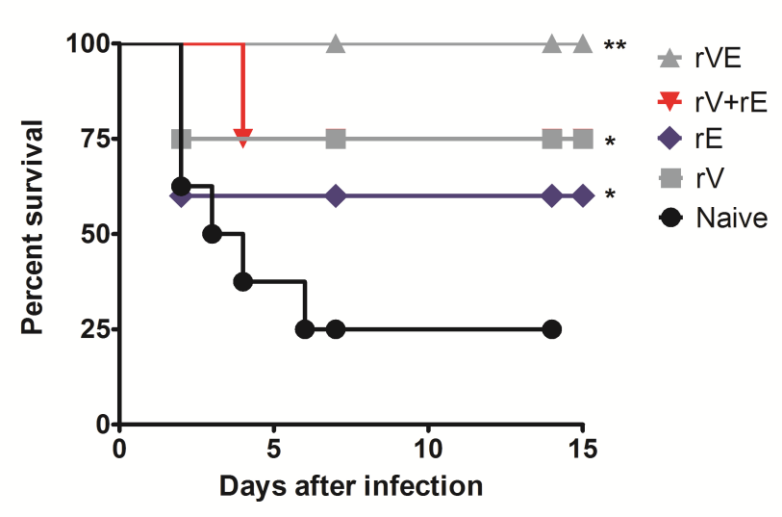


Prevention of actin filament perturbation and cell death against *Y. enterocolitica* O:8 infection.

Results

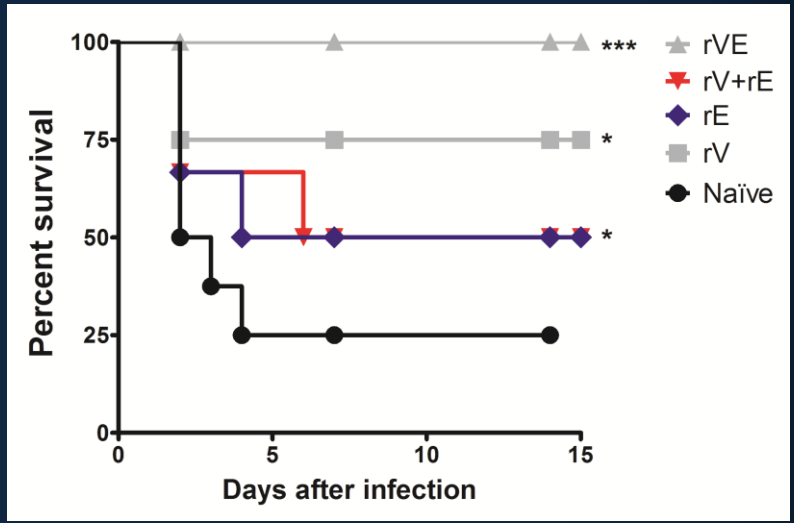
In-vivo protective efficacy

Active protection study



Kaplan Meier graph showing percentage survival in rV, rE, rV+rE and rVE group of immunized BALB/c mice I.P. challenge with *Y. enterocolitica*

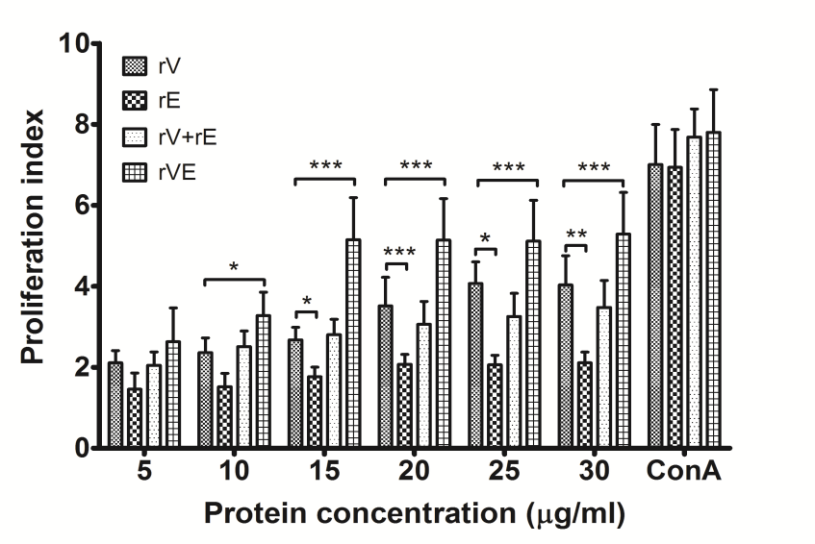
Passive protection study



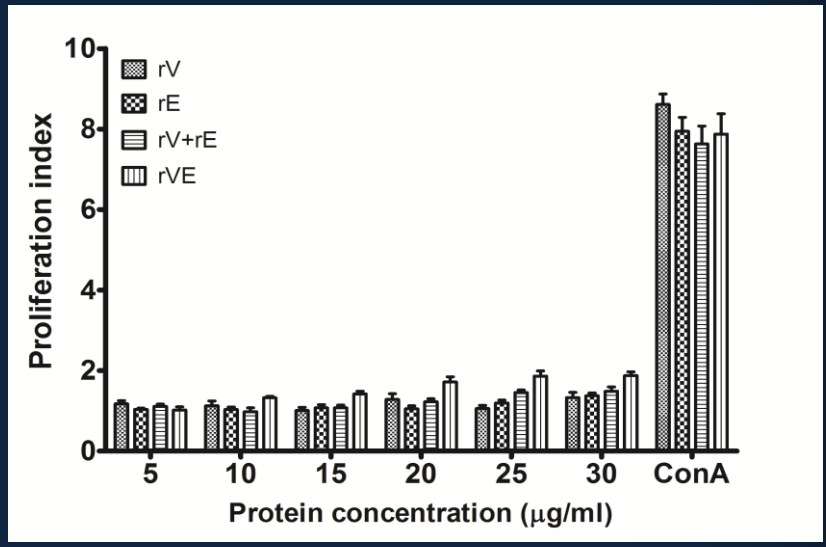
Kaplan Meier graph showing percentage survival in anti-rV, anti-rE, anti-rV+rE and anti-rVE group of immunized BALB/c mice I.P. challenge with *Y. enterocolitica*

Results

Lymphocyte Proliferation Assay



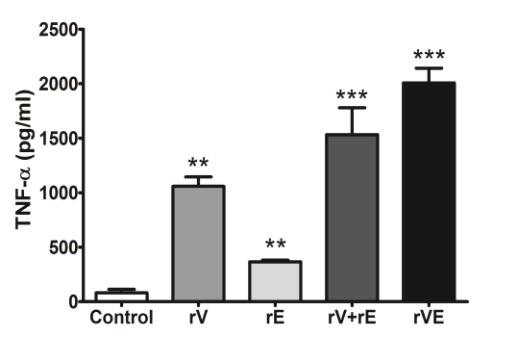
In-vitro proliferation of rV, rE, rV+rE and rVE primed splenocytes induced with their respective purified proteins (rV, rE, rV+rE and rVE) for 72 h at 37 °C

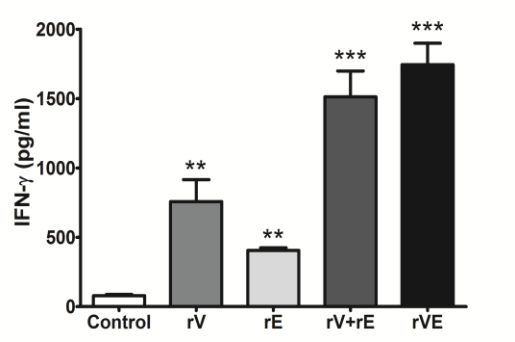


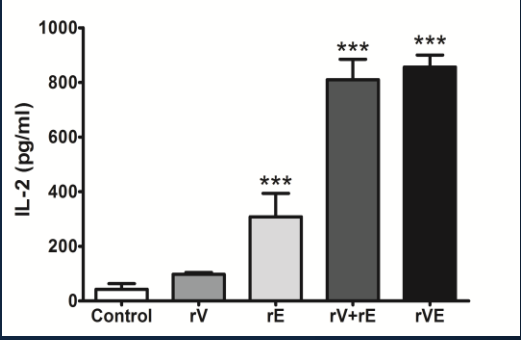
Sham Immunized mice splenocytes induced with purified proteins rV, rE, rV+rE and rVE for 72 h at 37 °C

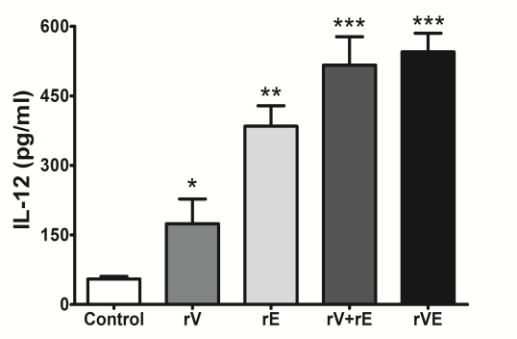
Results

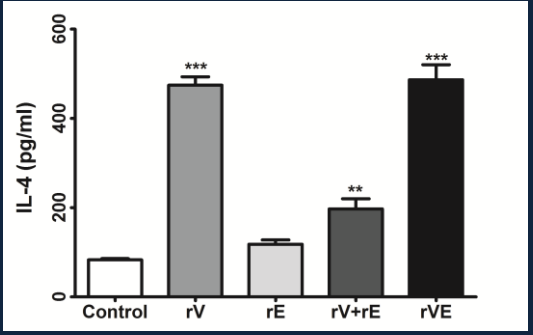
Cytokine profile

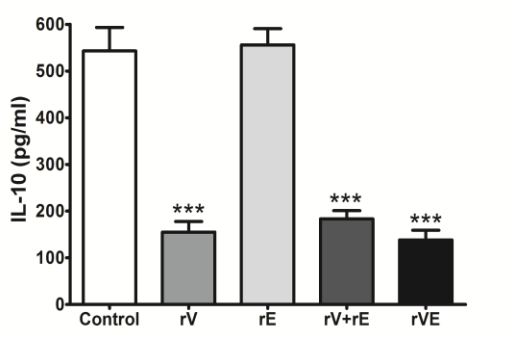








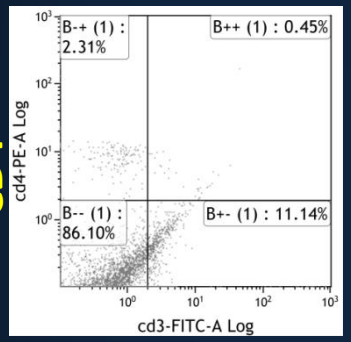


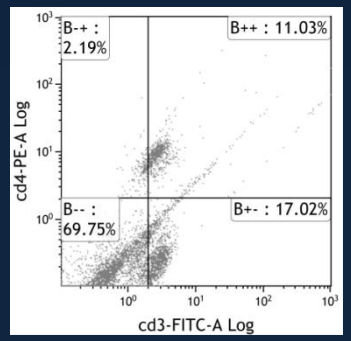


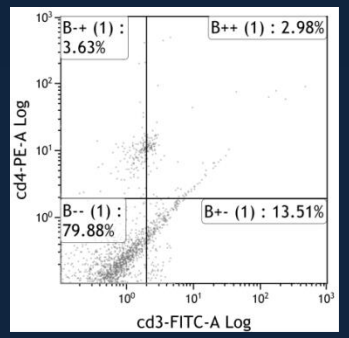
Results

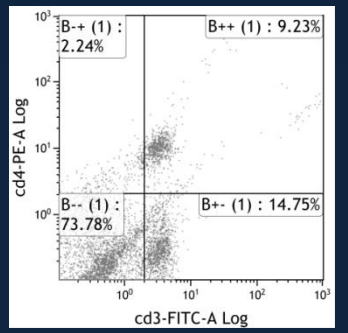
CD4 and CD8 T-cell response

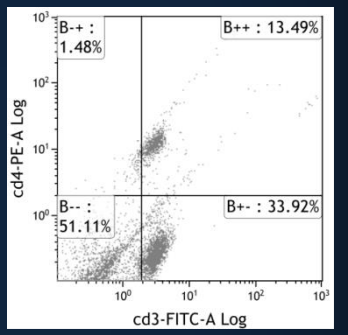
CD4



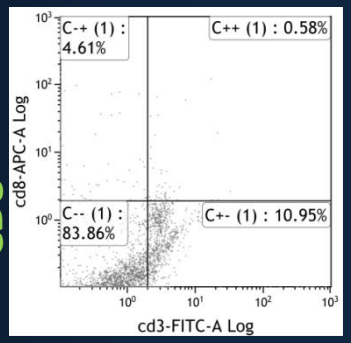


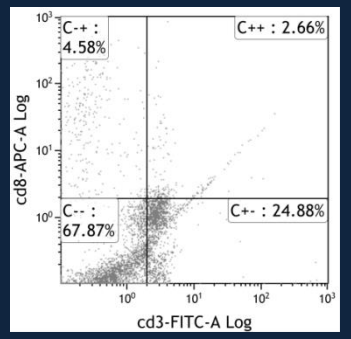


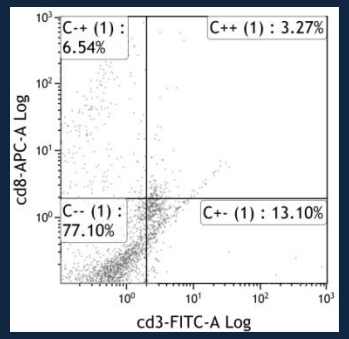


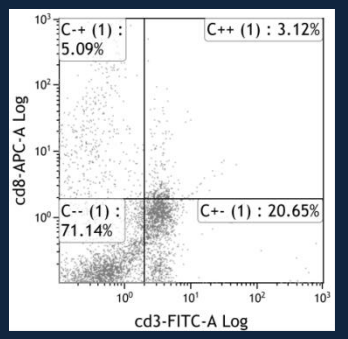


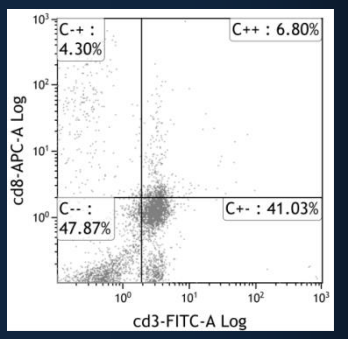
CD8







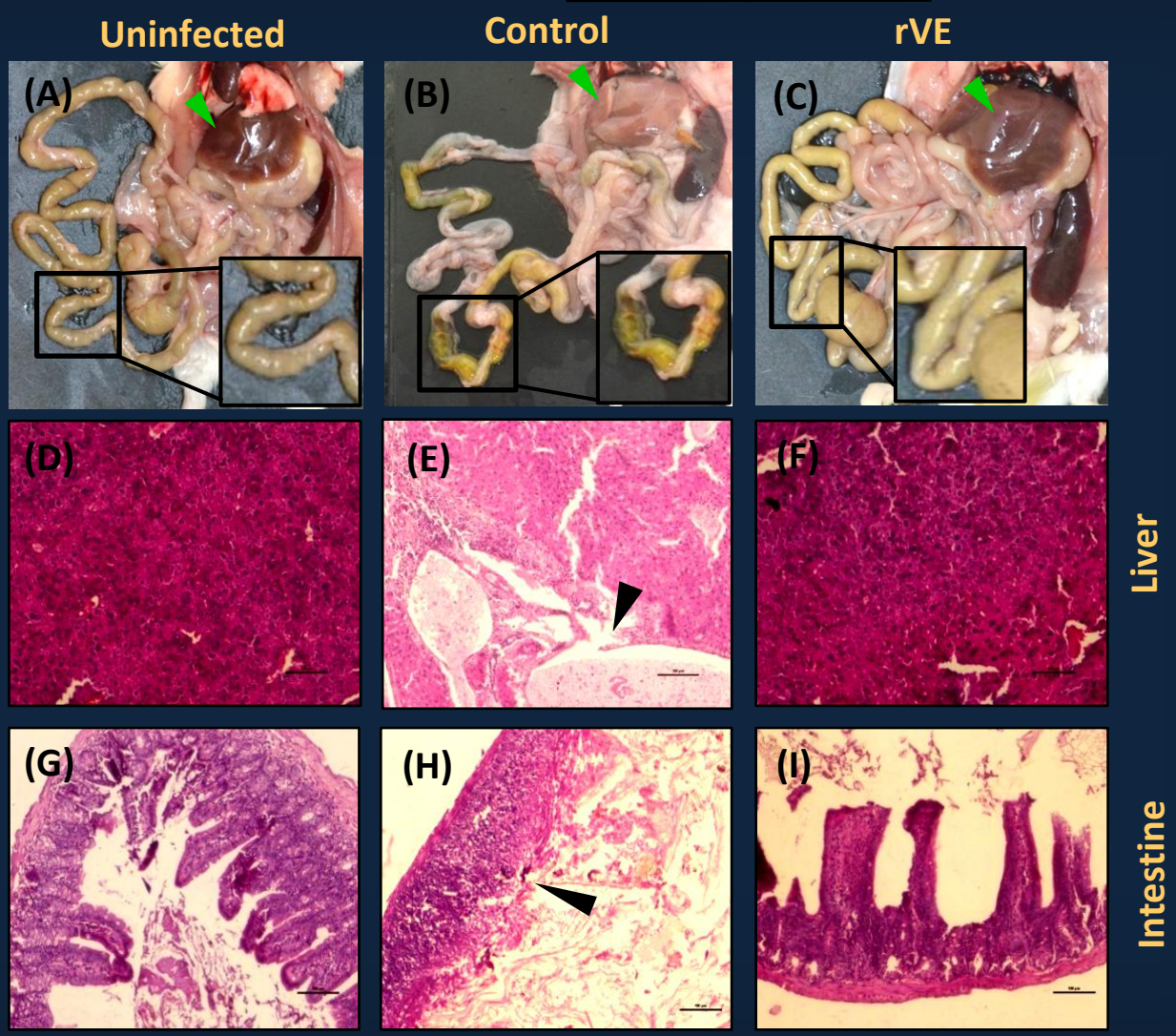




Sham rV rE rV+rE rVE

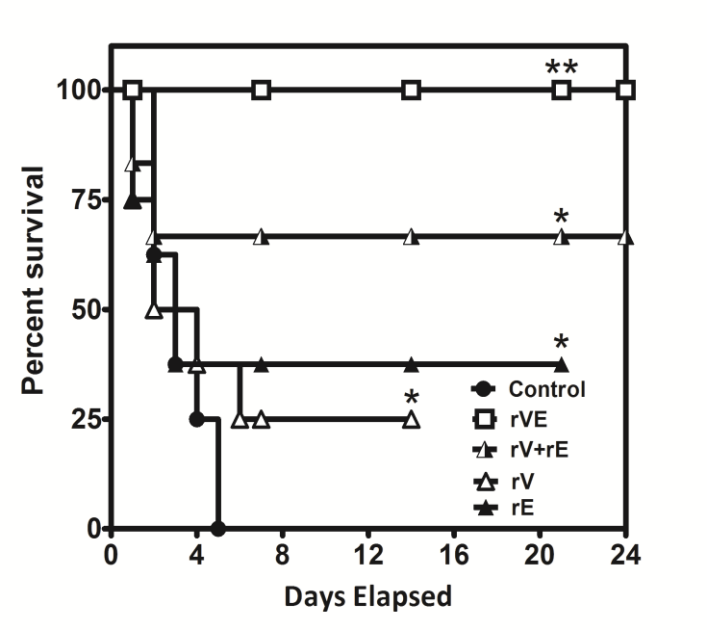
Results

Gross Pathology and Histopathology



Results

Memory response



Kaplan Meier survival curve of sham immunized and immunized groups of mice I.P. challenged with *Y. enterocolitica* after 120days post immunization

Salient Findings

- Developed a novel bivalent recombinant fusion protein (rVE) comprising of immunologically active regions of *Y. pestis* LcrV and YopE protein with an intervening 15 amino acids flexible glycine linker $(G_4S)_3$.
- Immunization with purified rVE protein developed robust and strong humoral immune responses mounted with a prominent IgG1 and IgG2a/IgG2b response in BALB/c mice.
- A significantly higher in-vitro lymphocyte proliferation at 15 µg/ml concentration of rVE protein.
- Complete protection to rVE group of mice against lethal *Y. enterocolitica* challenge in both active and passive immunization modules.
- CD4+ and CD8+ T-cell mediated long term protection maintained upto 4 months post immunization.
- Petite or no conspicuous sign of infection in gross pathology and histopathological analysis of spleen, liver and intestine of anti-rVE immunized mice.

Acknowledgements

- Parents
- Dr. H. V. Batra, Director, DFRL, Mysore.
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