STUDIES ON MOLECULAR PATHOGENESIS OF *PESTE DES PETITS* RUMINANTS (PPR) VIRUS

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Understanding the Nature of Morbillivirus Disease

Animal health & Human health

⇓

complimentary to each other

⇓

Well-being of animal population

⇓

An important ingredient & determinant of human health

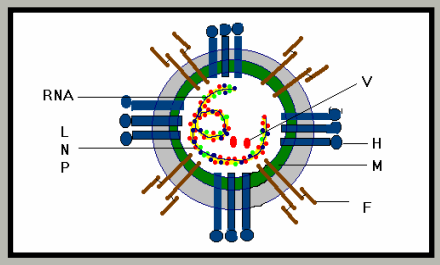
The Core Interest

- Pathogens long present but not previously identified
- Recognition of apparently new microbes
- Changes in epidemiology (changes in distribution, disease incidence, virulence of microbial strains, microbial resistance to drugs) of old pathogens
- New disease—disease interactions and
- Spread (to human/animals) of organisms never previously known to be human/animal pathogens

Peste Des Petits Ruminants (PPR)

- **Economically important disease of Small ruminants (Goat and Sheep)**
- **Caused by PPR virus:**

  - Negative sense, Non-segmented, Single stranded RNA



Taxonomy:

Order: *Mononegavirales*

Family: Paramyxoviridae

Subfamily: Paramyxovirinae

Genus: Morbillivirus

Members:

(i) Measles virus (Type species)

(ii) PPR virus

(iii) Rinderpest virus

(iv) Canine distemper virus

(v) Phocine distemper virus

(vi) Dolphin and Porpoise (Cetacean) Morbilliviruses

Major Areas of Investigations

- Receptor based studies on PPR virus:
  - Understanding viral choice for an epithelial (CD46) and a lymphocyte receptor (CD150)
- Distribution of PPR antigen in various components of blood in vaccinated and infected animals:
  - Virus sequestration and localization as key factors to generation of variant
- *in vivo* cytokine expression profile in vaccinated and infected animals:
  - Role of soluble factors in immune response

Part I: The Receptor Based Study

1. **PCR amplification of SLAM* gene from mitogen stimulated caprine PBMC**

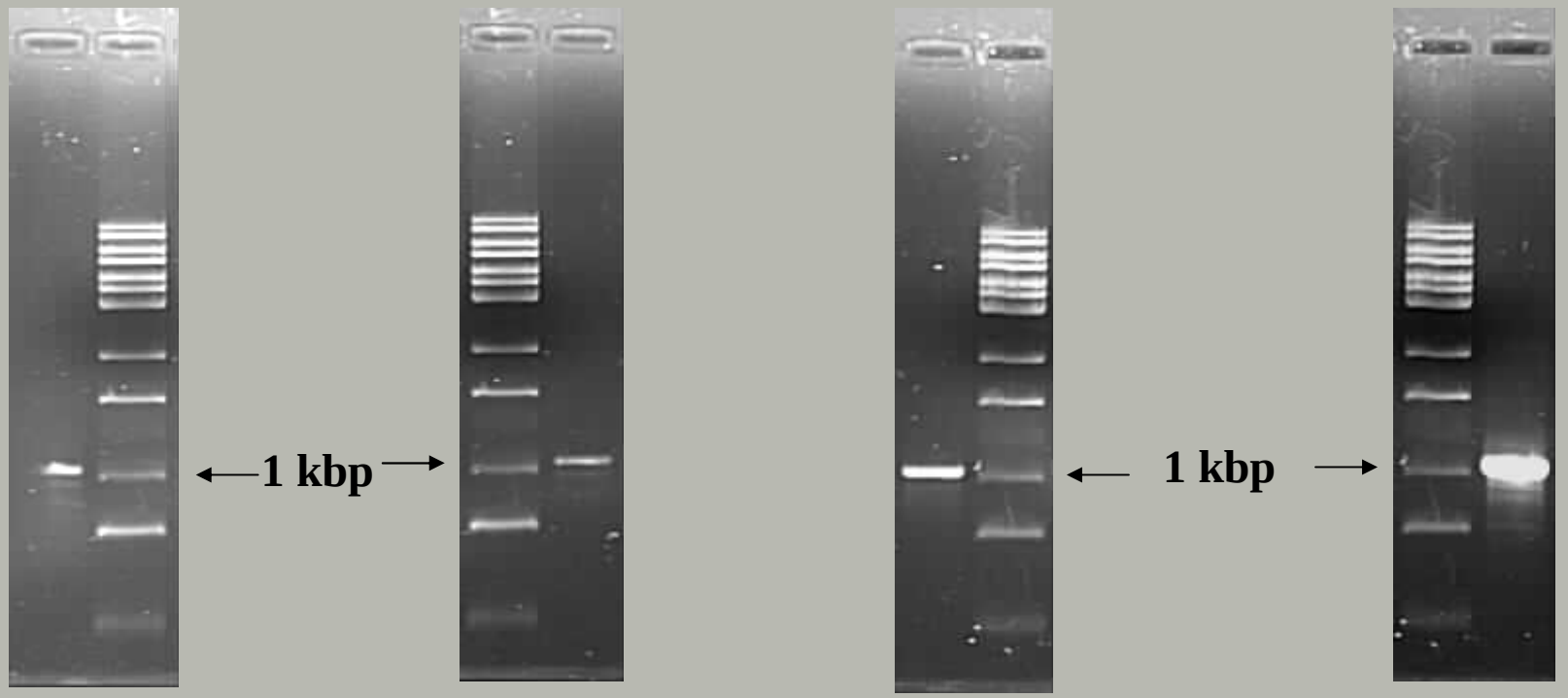
2. **Cloning and subsequent sequencing of Caprine SLAM gene**

3. **Development of a stable Vero cell line(Vero/SLAM) constitutively expressing caprine SLAM* along with CD46**

4. **Comparing susceptibility of PPRV to Vero/SLAM with Normal Vero cells**

*** Signaling lymphocyte activation molecule (CD150)**

PCR amplified SLAM genes *from PBMC'S*



Sheep **Goat** **Buffalo** **Cattle**

Size of amplicon is 1017 bp

Endotoxin free recombinant pTarget Vector containing caprine SLAM gene was isolated.

↓

Vero cells were transfected with the endotoxin free recombinant vector keeping control wells (without transfection).

↓

Cultured at 37ºC for 48h.

↓

The cells (including control wells) were subjected to G418 selection pressure .

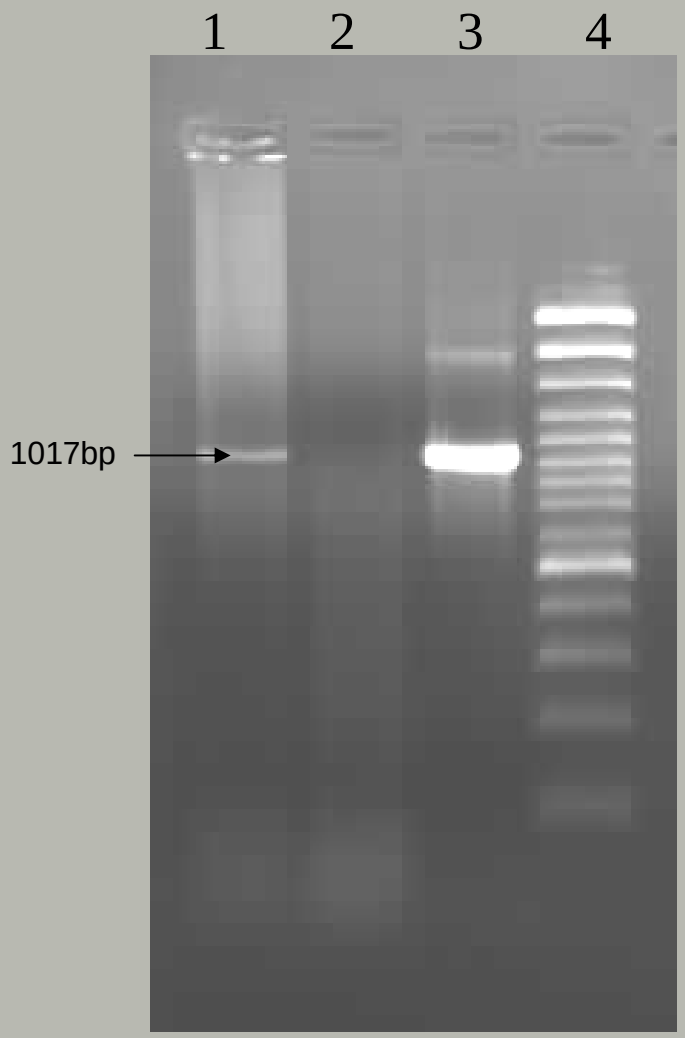
↓

The cells were monitored everyday.

↓

On 8^{th} day after selection,all the cells in control wells died;whereas there were selected few live cell in transfected wells scattered throughout the plate.

Determining integration of caprine SLAM gene into the genome of Vero/SLAM cells



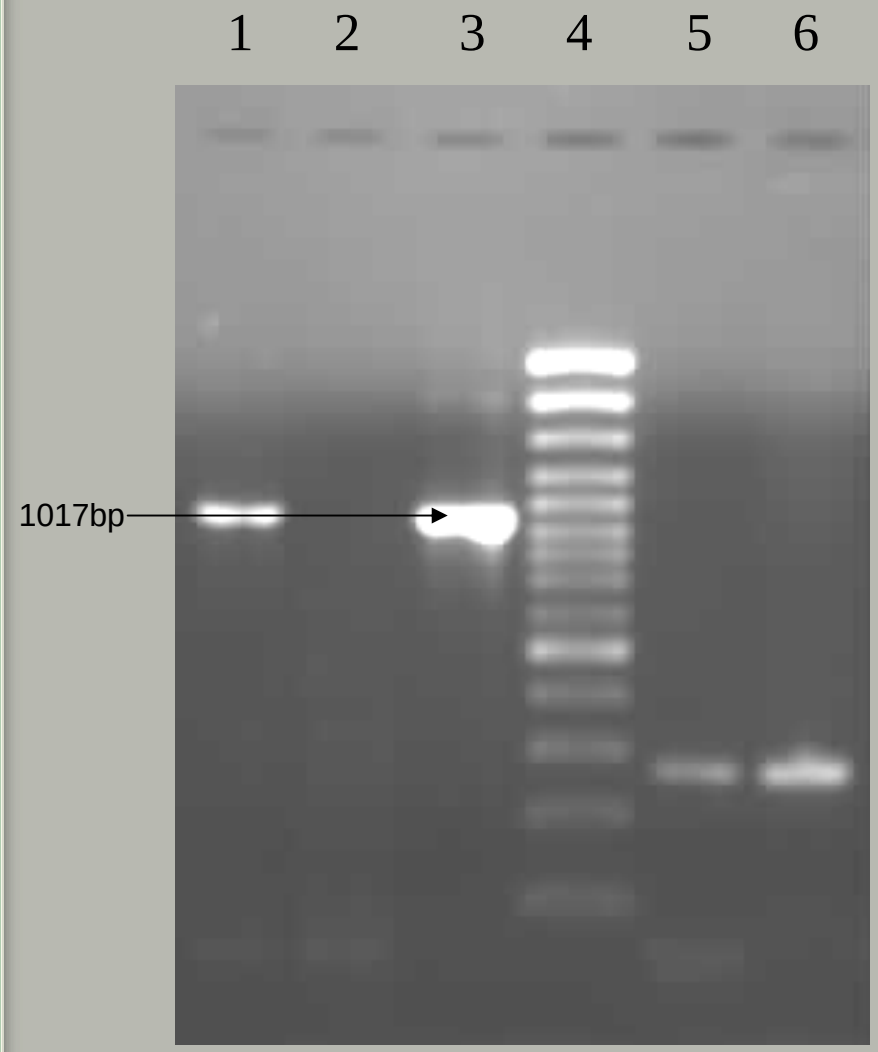
Lane 1: PCR amplicon from Vero/SLAM genomic DNA

Lane 2: PCR amplicon from Vero genomic DNA

Lane 3: Positive control (pTARGET cloned plasmid)

Lane 4: 100bp plus DNA ladder

Determining expression of caprine SLAM in Vero/SLAM cells



SLAM/BT/for&rev

Lane-1: PCR amplicons from Vero/SLAM cell line using SLAM/BT/(for&rev) primers

Lane-2: PCR amplicons from Vero cell line using SLAM/BT/(for&rev) primers

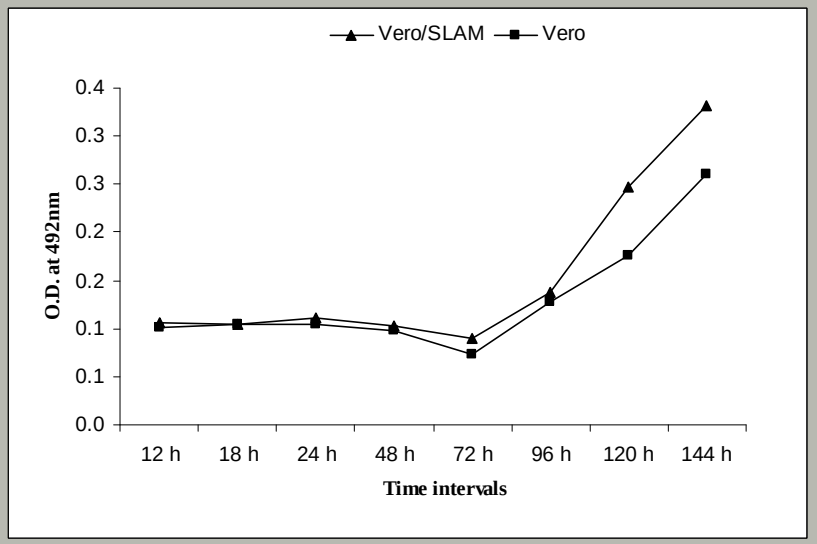
Lane-3: Positive control (PCR amplification of pTARGET cloned plasmid with SLAM/BT/ (for & rev) primers

Lane-4: GeneRulerTM 100bp DNA Ladder Plus (MBI, Fermentas, Cat#SM0321)

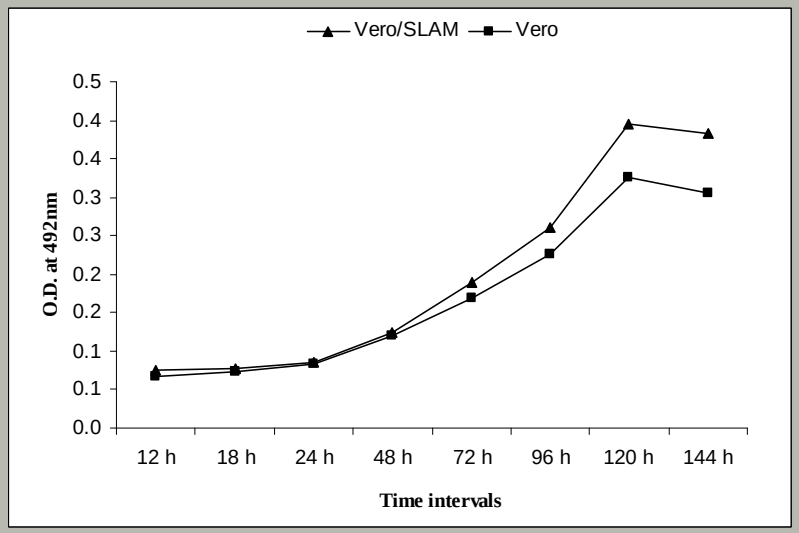
Lane-5: PCR amplicons from Vero/SLAM cell line using BA1&BA2 primers

Lane-6: PCR amplicons from Vero cell line using BA1&BA2 primers.

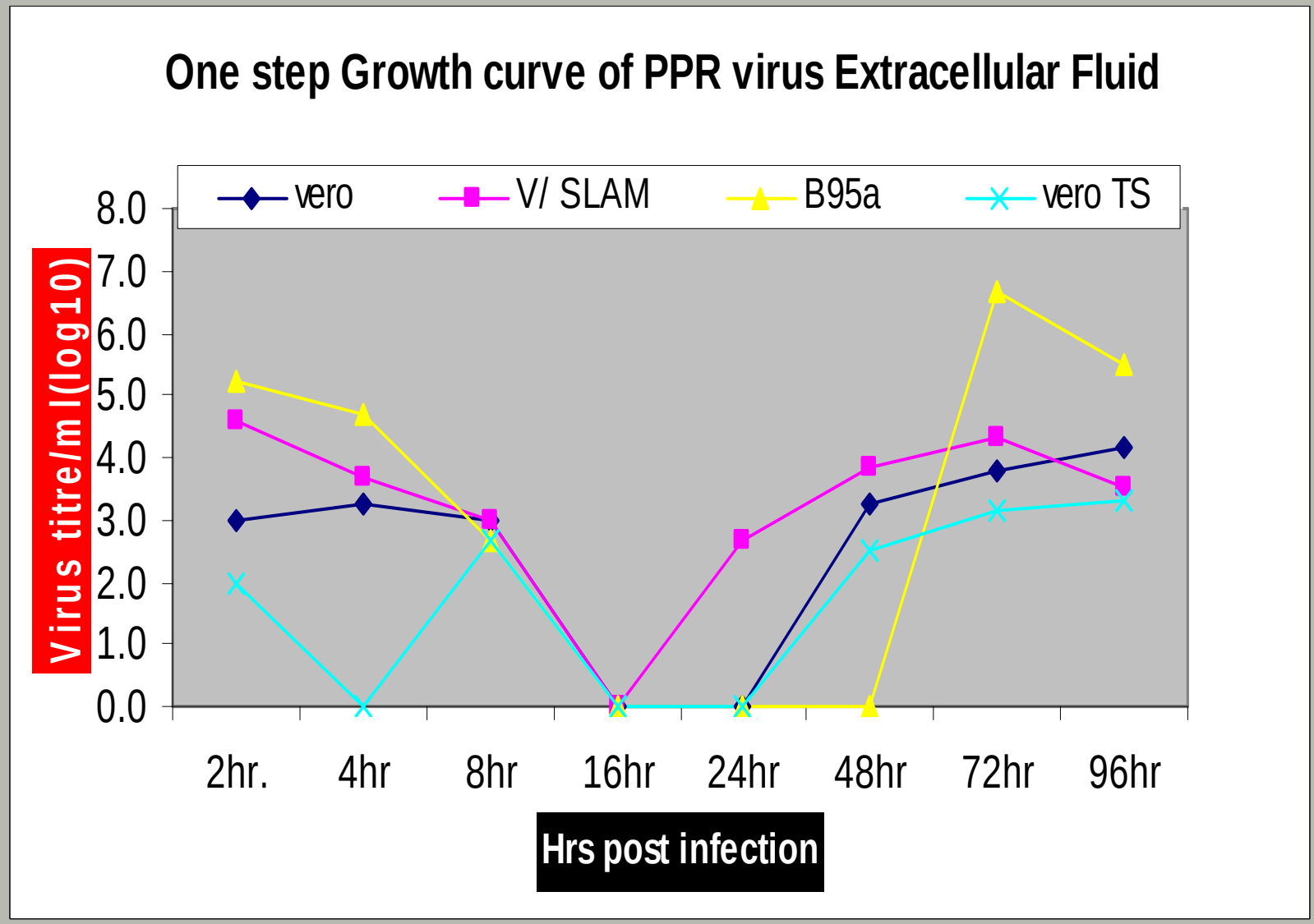
Comparative viral load, measured by sandwich ELISA at various time intervals in Vero/SLAM and Vero cell lines infected with PPR vaccine virus



Cell Supernatant



Intracellular virus


One step Growth curve of PPR virus Extracellular Fluid
vero
V/ SLAM
B95a
vero TS
Virus titre/ml(log10)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
2hr.
4hr
8hr
16hr
24hr
48hr
72hr
96hr
Hrs post infection

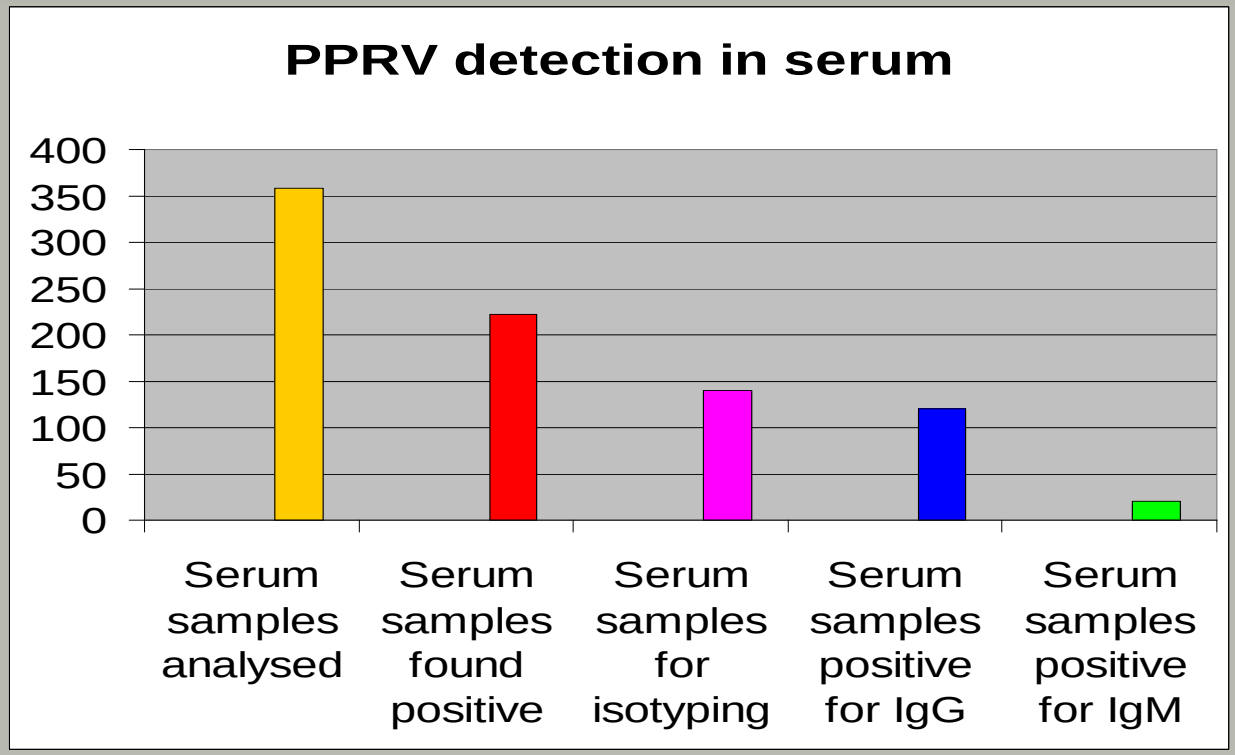
Other receptor(s)/co-receptor(s)?

- Due to error-prone nature of RNA genome, *Morbilliviruses* have a high mutation rate and can easily adapt to use an alternate receptor(s) *in vitro*
- Since SLAM-positive cells are a minor population in the body, it may be possible that PPR virus has evolved (or evolving?) using an alternate receptor for entry into the host
- "From this work it has been possible to clearly demonstrate that SLAM is definitely a high affinity receptor for the PPR vaccine virus. A novel and stable transfected Vero cell line displaying SLAM on its surface has been developed. Possibility of existence of other molecule(s) acting as principle cellular receptor(s)/co-receptor(s) cannot be ignored."

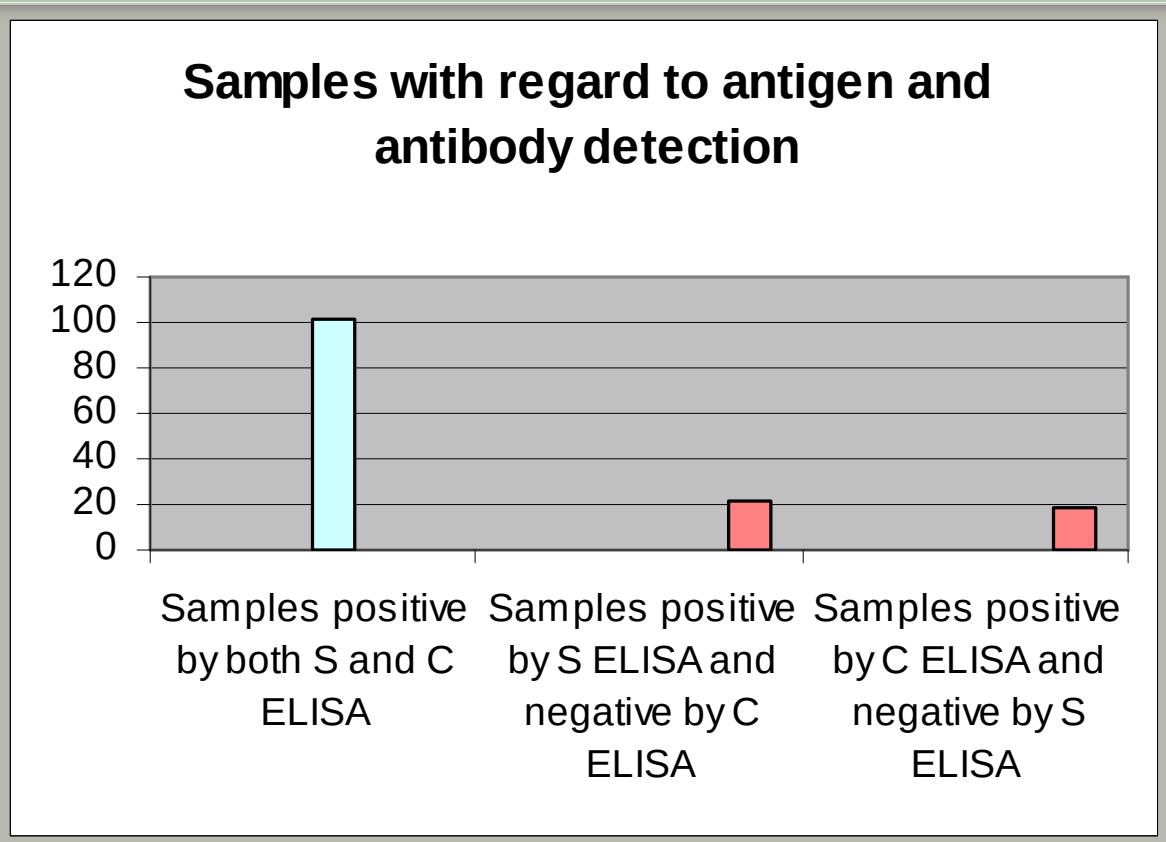
Part IIa: Studies on presence of PPRV in serum
(The studies that were carried out in this project)

Detection of PPRV antigen in serum

- **Isotyping of serum samples to assess the nature of immune response**
- **Detection of PPR viral antigen in various components of blood**
- **Evaluation of cytokine response in PPR infected and vaccinated animals**
- **Evaluation of apoptosis in PBMC's from PPR infected goats**

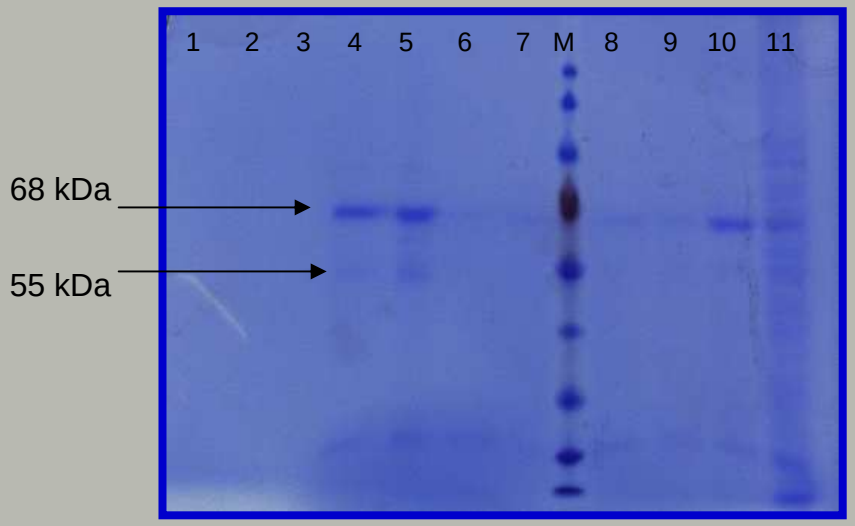


1	Serum Samples analyzed	358 (275 goats+83 sheep)
2	Serum samples found positive for PPRV antigen	222 (62.01%- Overall (70.54% goats +33.73% sheep)
3	Serum samples isotyped	140 (random)
4	Serum samples positive for IgG	120 (85%)
5	Serum samples positive for IgM	20 (15%)



Samples positive by s ELISA and c ELISA	101
Samples positive by s ELISA but negative by c ELISA ******could signify persistence or antigenic variants.**	21****
Samples positive by c ELISA but negative by s ELISA	18

Immunoprecipitation of serum samples positive for PPR viral antigen



- Red band in M lane – 72 kDa fermentas prestained markers
- With polyclonal two bands are seen corresponding to 68kda (H protein) and 55kda (N protein)
- With monoclonal only one band at 68 kda is seen.

1, 2, 3 : negative serum

4, 5 : positive serum immunoprecipitated with polyclonal antibodies

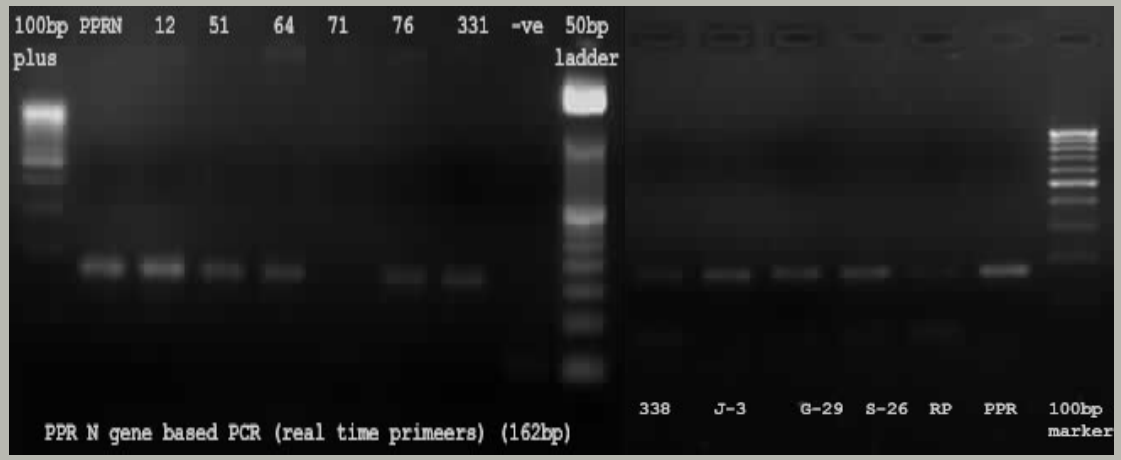
6, 7, 8, 9 : positive serum immunoprecipitated with anti H monoclonal antibodies

10 : infected cell lysate immunoprecipitated with polyclonal antibodies

11 : infected cell lysate

Serum samples were precleared extensively by equilibrated agarose before immunoprecipitaing with rabbit HIS or with monoclonal antibody.

Detection of PPRV “N” and PPRV “M” gene in serum samples by PCR



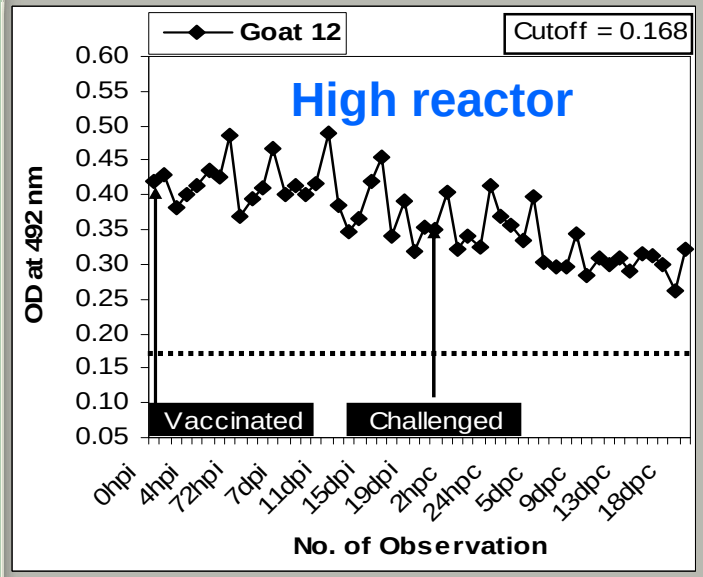
PPRN gene based PCR (162 bp product)

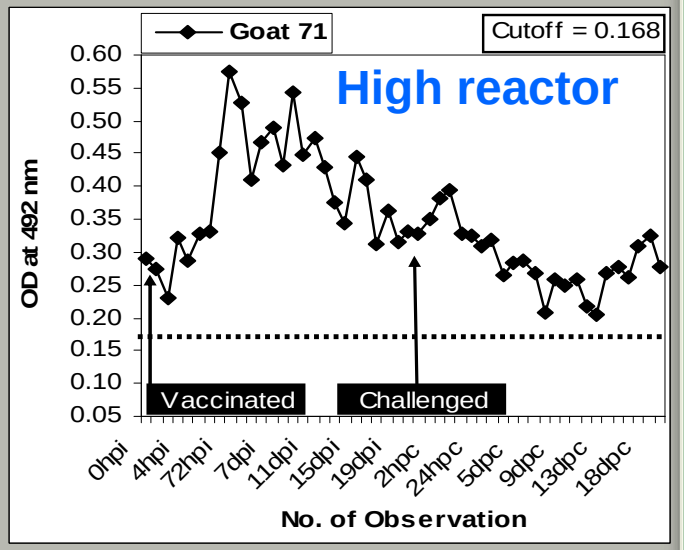
PPRM gene based PCR (124 bp product

Part IIb: Detection of PPR Viral Antigen in Various Components of Blood: Some Critical Findings

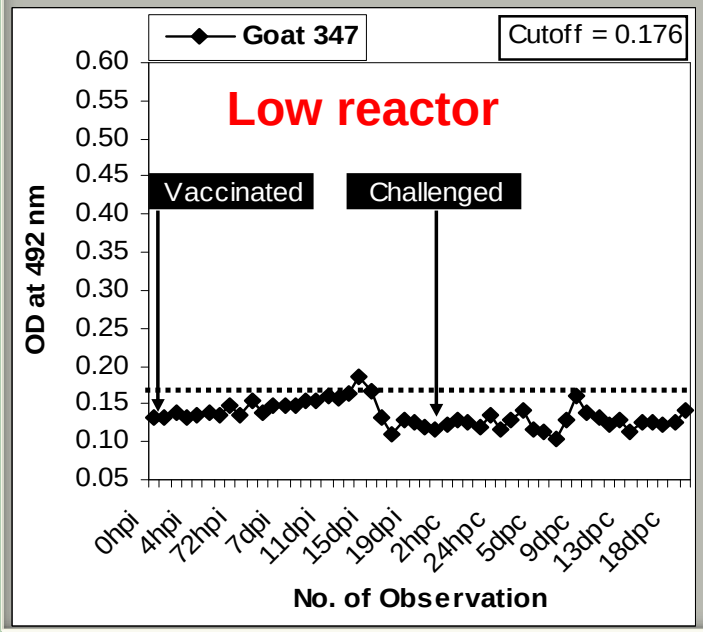
- **Viral load was more in plasma (almost > 1.5 times) than whole blood**
- **Challenged animals exhibited high level of PPRV antigen titer [from 8-9th day post challenge and persisted up to 11th day post challenge or till the animal survived the disease] than vaccinated ones**
- **Some animals showed positive reaction for PPRV from 0 day onward, indicating possible viral persistence**
- **PBMCs showed negative reaction for PPRV in s ELISA in all animals**
- **Viral antigen was detected in PBMCs of PPR infected goats from 72 hrs to 11th day post infection by Q-PCR using SYBR green dye chemistry**

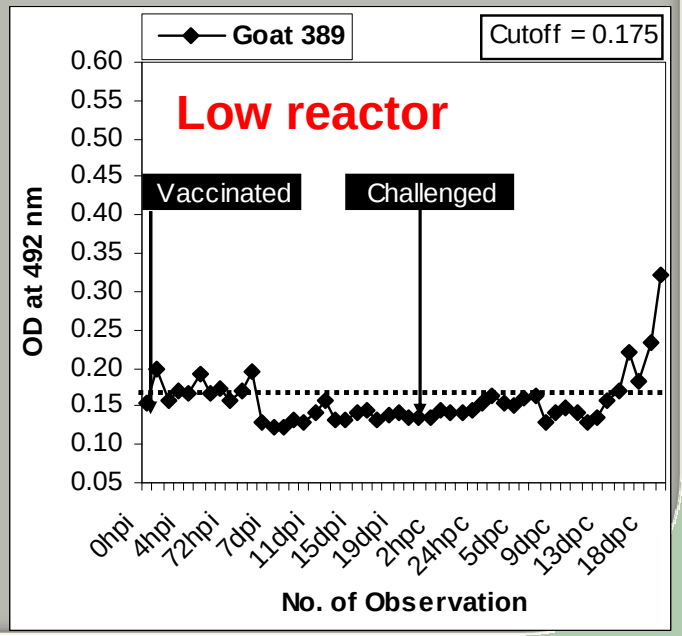
Detection of PPRV Antigen In Plasma of Vaccinated Followed by Challenge Animal by sELISA



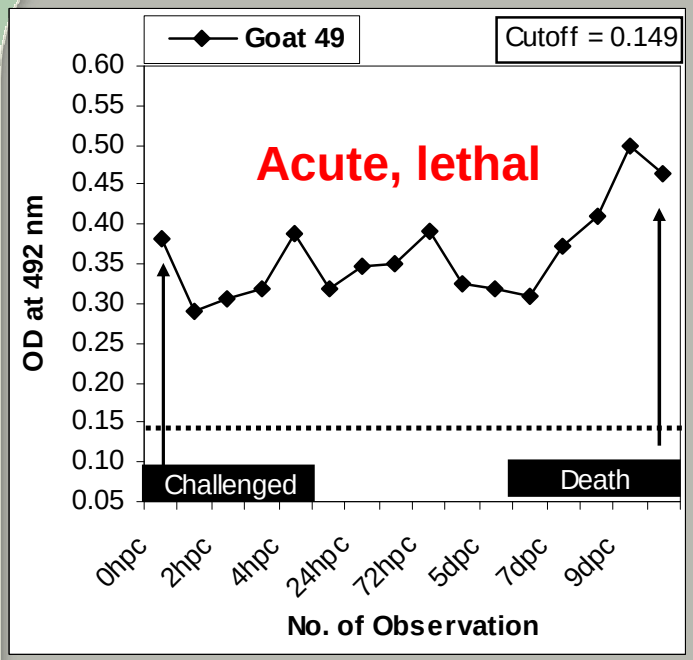


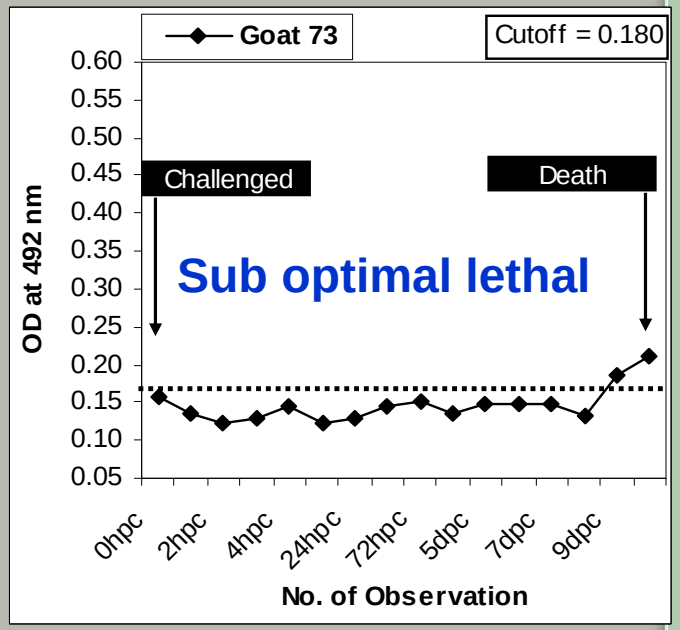
Plasma

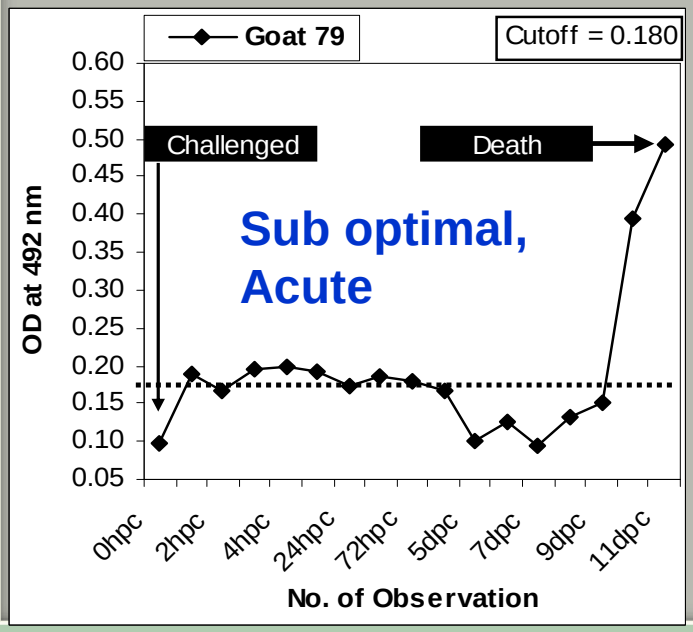


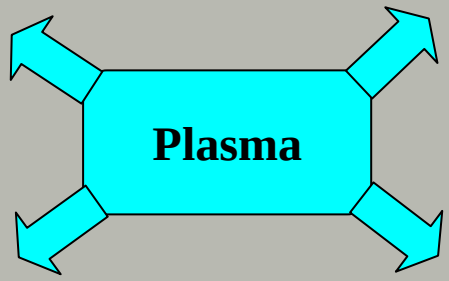


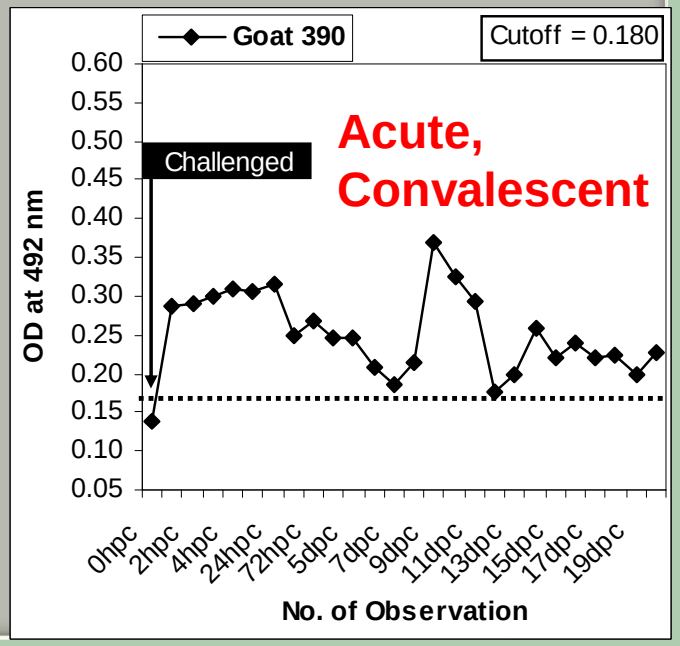
Detection of PPRV Antigen In Plasma of Challenged Animal by sELISA



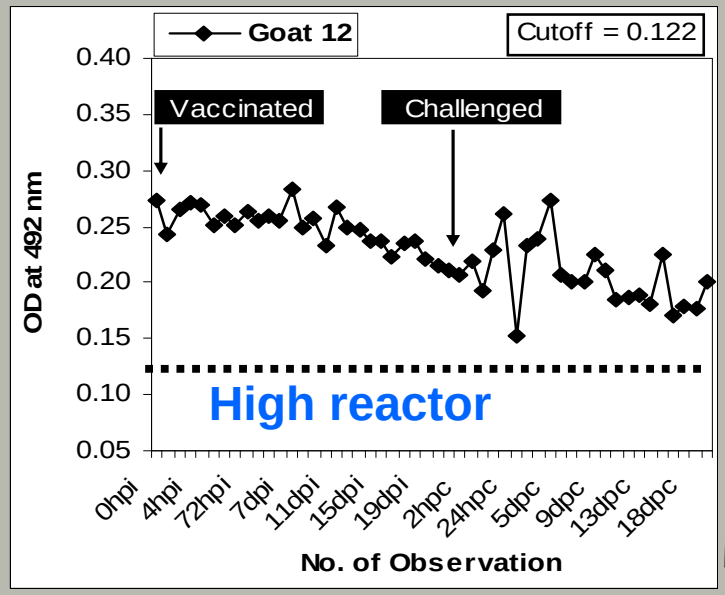


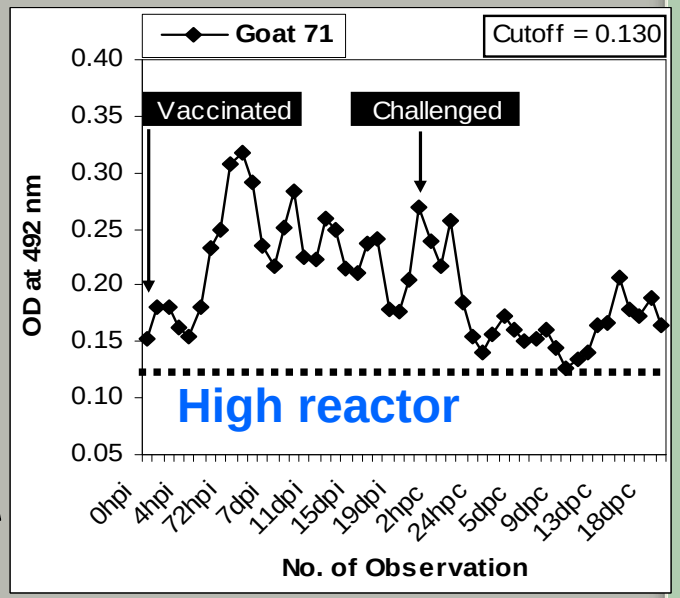




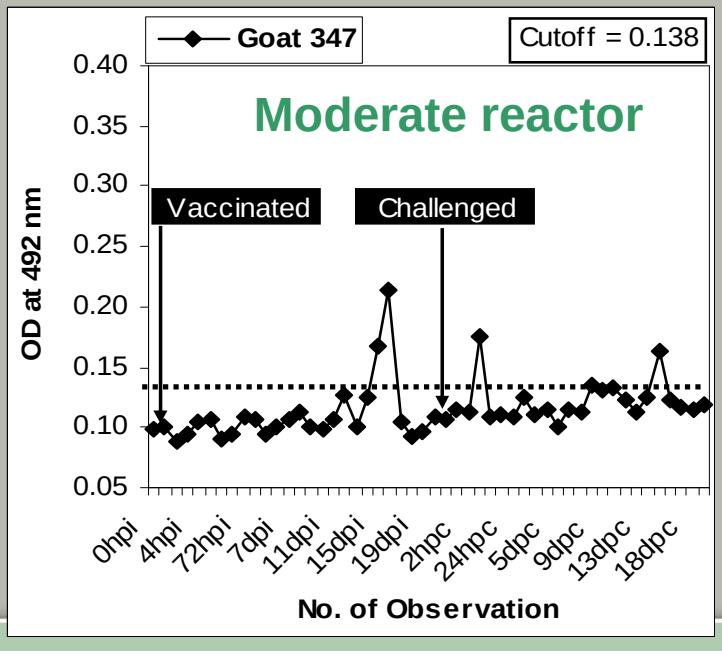


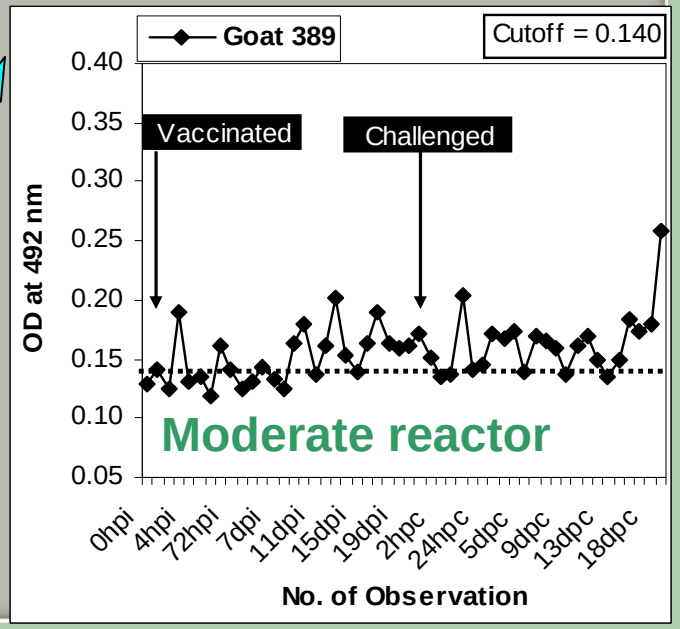
Detection of PPRV Antigen In Whole Blood of Vaccinated Followed by Challenge Animal by sELISA



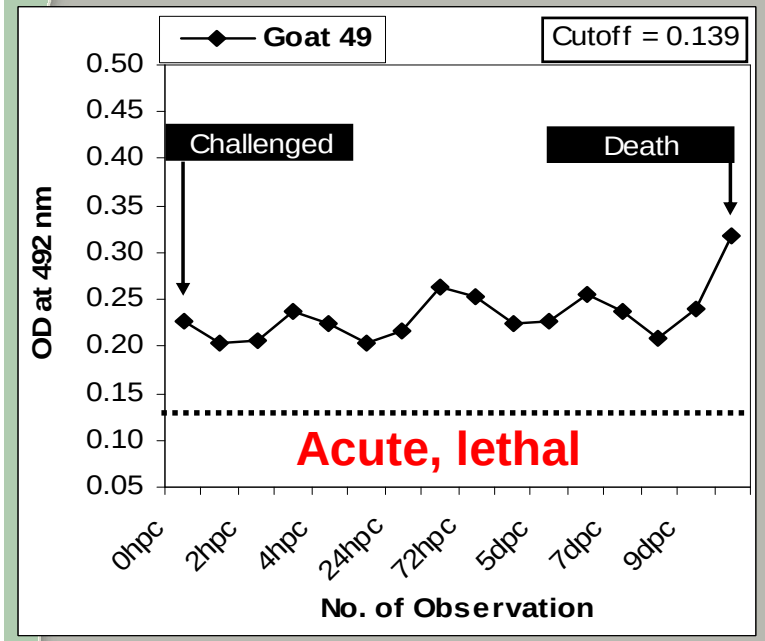


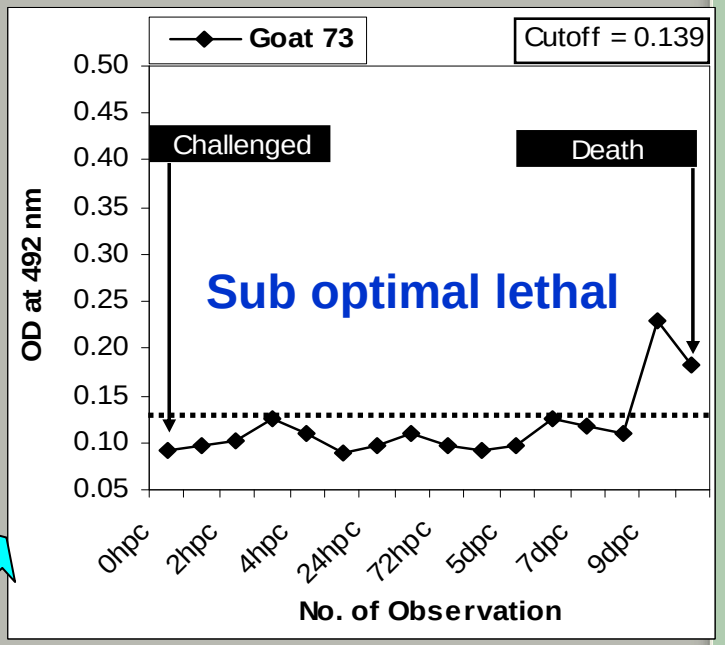
Whole Blood



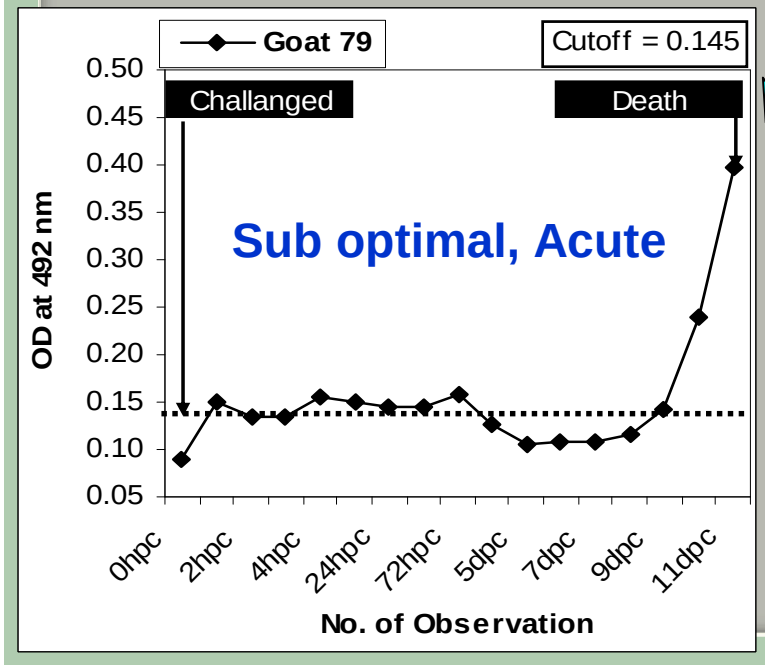


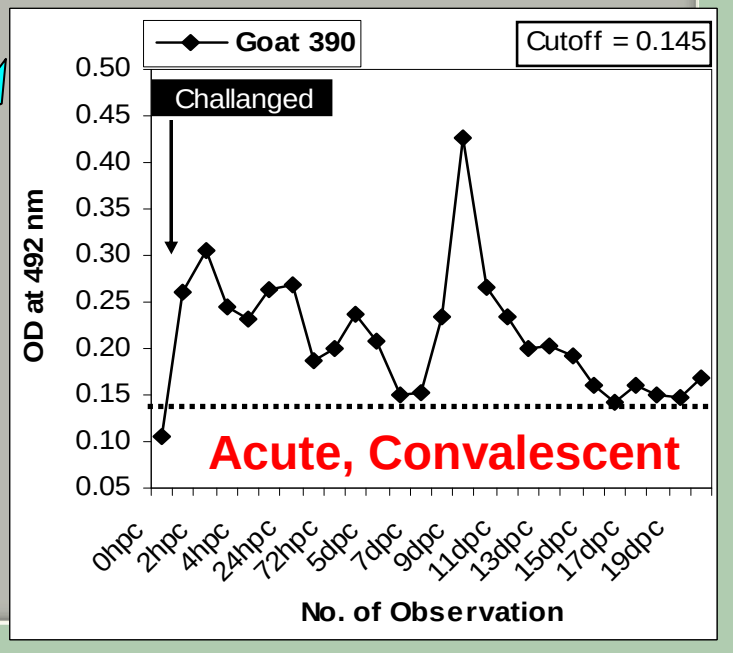
Detection of PPRV Antigen In Whole Blood of Challenged Animal by sELISA





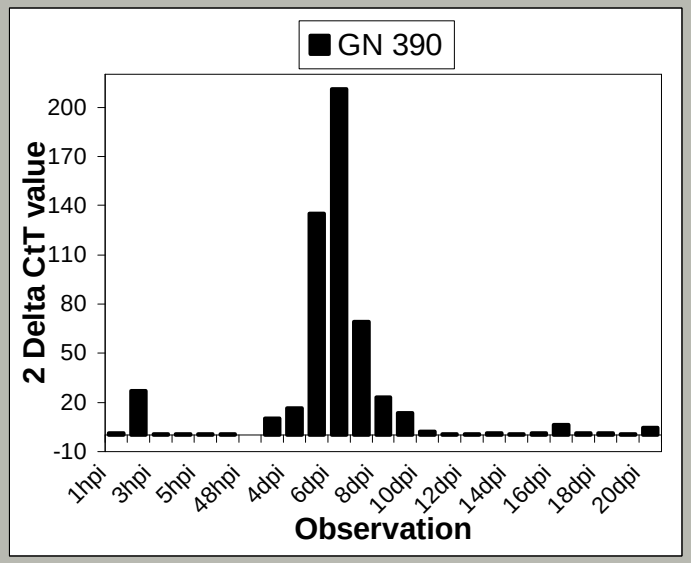
Whole Blood

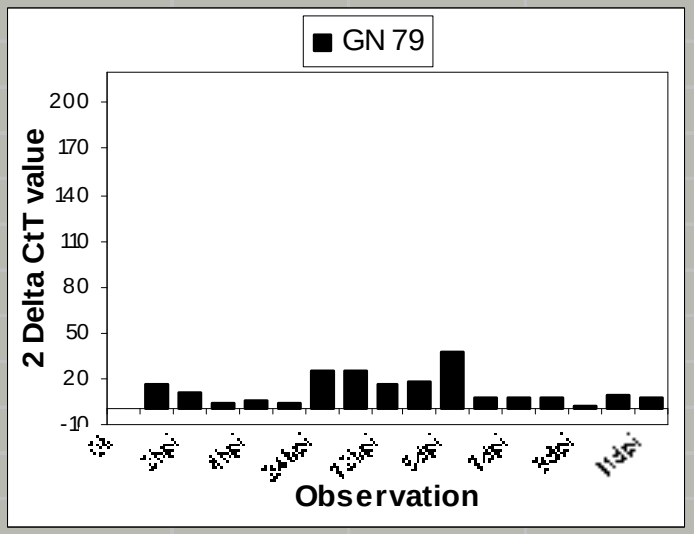




Detection of PPRV Antigen in PBMCs of Challenged Animal by Q-PCR Using SYBR Green Dye

PPR N-Gene





Virus detected in unvaccinated challenged animals from 72 hours to 11 days post challenge

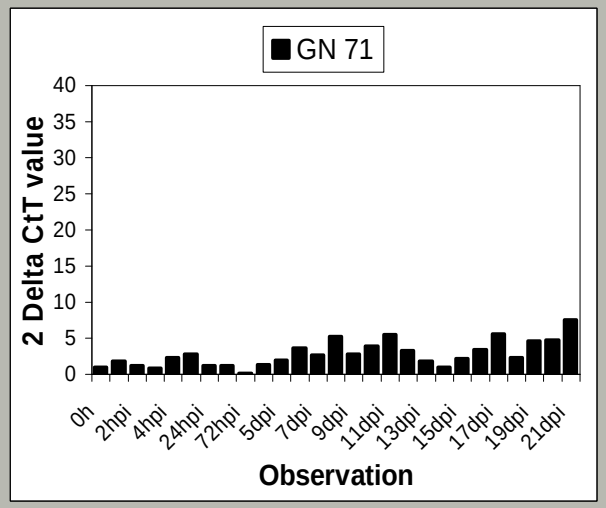
Part III: *In vivo* Cytokine Expression Profile

- Two important cytokine genes were studied for their modulation by real time PCR using PBMCs from vaccinated and challenged animals.

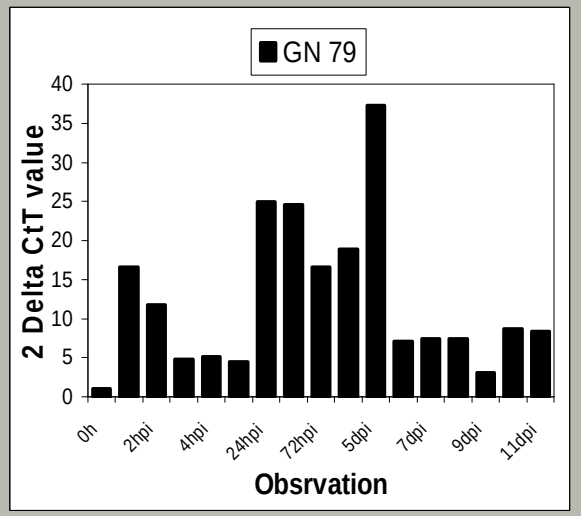
1. Interferon Gamma
2. Interleukin 4

Interferon Gamma (IFN-γ) Gene

- **Expression of IFN-γ in vaccinated animals was less pronounced in comparison to infected animals**
- **Early expression was seen in challenged animals than vaccinated ones**



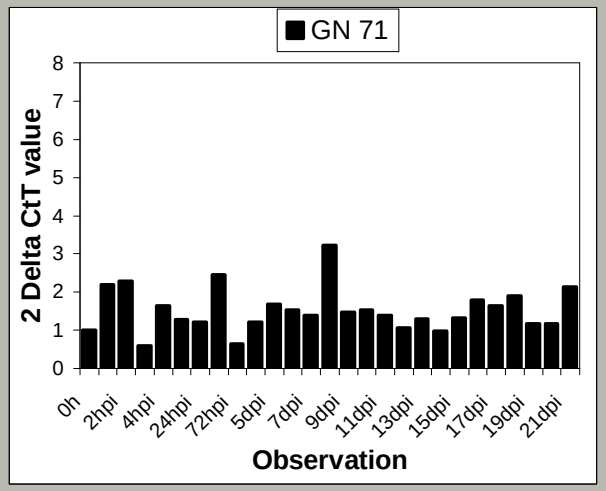
Vaccinated



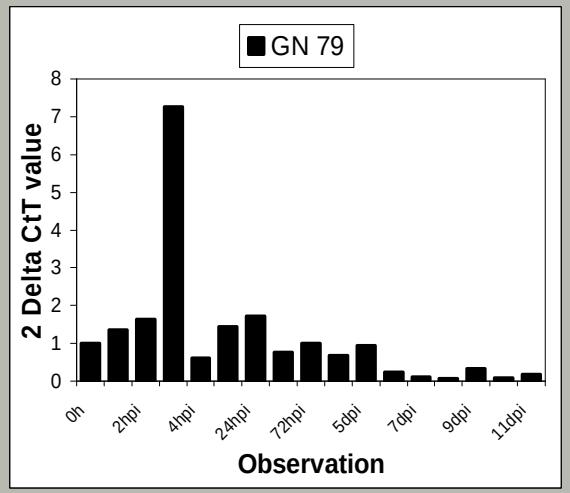
Challenged

Interleukin-4 (IL-4) Gene

- **Marked difference experienced between vaccinated and challenged groups**
- **Down-regulation seen in challenged animals (4dpc-15dpc/till death)**
- **No much variation in expression in vaccinated animals (steady throughout experiment)**



Vaccinated



Challenged

Salient Observations

- Plasma-in general-harbors more PPR virus than any other blood component
- PPRV can be detected in PBMCs by 3rd day post challenge by Q-PCR
- Cytokine picture reveals that:
  - Vaccinated animals showed Th2 response (Humoral) i.e.. ↑ IL-4
  - Challenged animals exhibited Th1 response (CMI) i.e.. ↓ IL-4
- PPRV antigen in serum could indicate either presence of immune complexes or persistence???
- Relevance of serum/blood/plasma borne PPR virus to its pathogenesis needs to be investigated.
- The role of sub-clinical PPR infection in atypical host???
- PPR along with related small ruminant infections need to be identified with regard to pathogenesis

Why Change in Disease Pattern?

- **Typically affected by interaction of multiple factors.**
- **The emergence/re-emergence of a disease ⇒ an unintended consequence of many developmental activities perceived as progress.**
- **The commonly recognized factors include:**

- **microbial adaptation and change**
- **human/animal demographics and behavior**
- **environmental changes**
- **technology and economic development**
- **breakdown in medical and veterinary public health measures**

and surveillance and

- **international travel and commerce**

(Lederberg et al., 1992)

‘PROSPEROUS – PEACEFUL – SAFE HUMAN HABITAT’

Delhi Sustainable Development Summit-2003

The most important need of the country is an integrated developmental plan and empowered management structures in the areas of Education and Healthcare, Agriculture and Food Processing, Information and Communication Technology, Strategic Sectors, Infrastructure Industries and Geo-politics & Ecology.

Dr. Abdul Kalam
Nuclear Scientist &
Former President of India

Thank You…..

Division of Virology

Since 1889

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