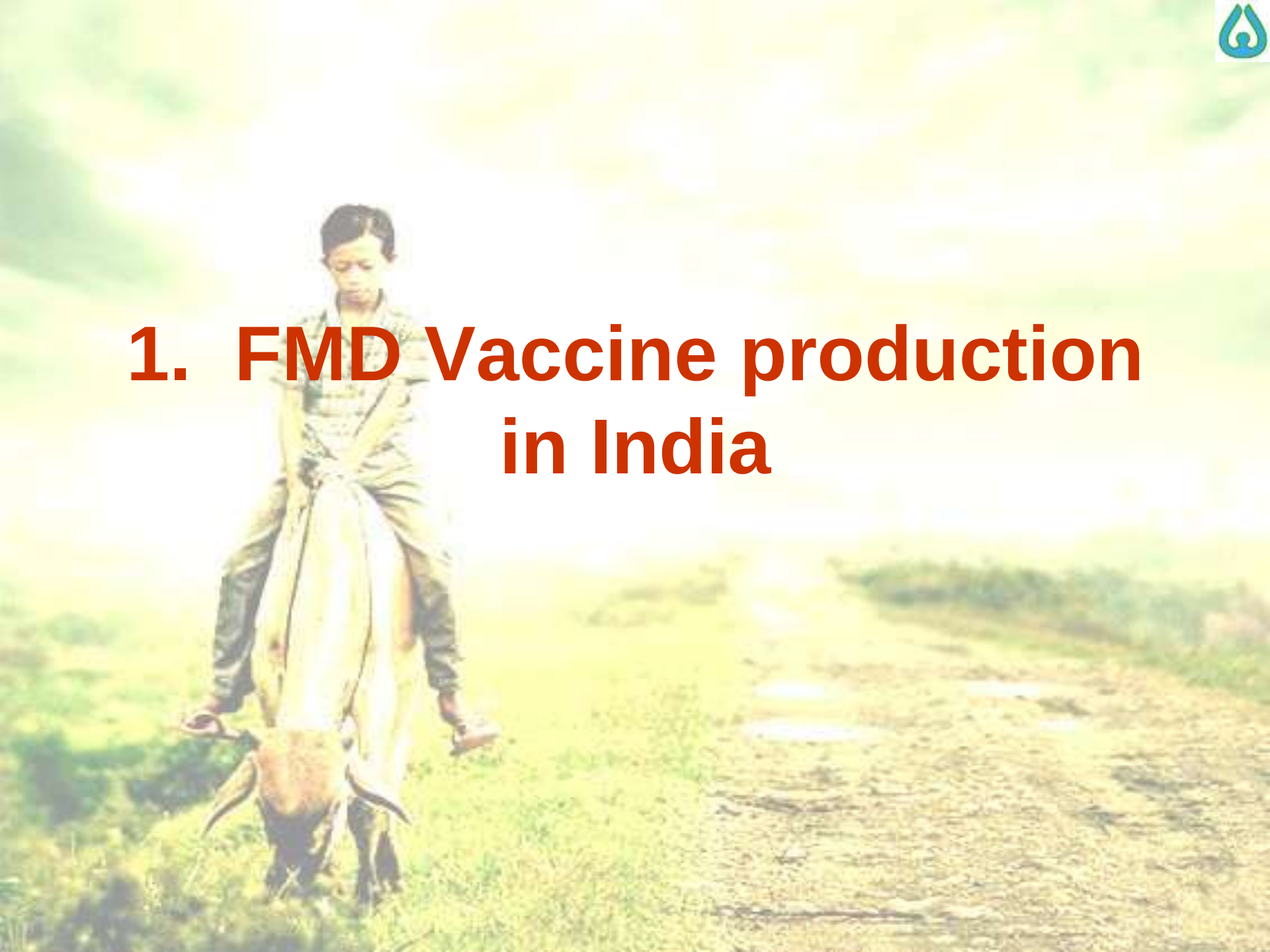
FMD vaccines and vaccination in India; Production, use and quality

V.A.Srinivasan
Research Director
Indian Immunologicals Limited
Hyderabad – 500 032

1. FMD vaccine production in India
2. Limitations in the inactivated vaccine
3. FMD Vaccine – Cold chain
4. FMD control programme
5. Recommendations



1. FMD Vaccine production in India

FMD vaccine production in India

1. FMD vaccine manufacturers
2. Vaccine production technology
3. Vaccine strain selection

FMD vaccine manufacturers

S.No.	Name of the Manufacturer	Installed Production capacity	Year established
1	Indian Immunologicals Limited	240	1982
2	IVRI - Indian Veterinary Research Institute	10	1972
3	BIOVET	40	2006
4	Brilliant Bio Pharma Limited	100	1998
5	Intervet India Pvt. Ltd (MSD Animal Health)	50	1975

FMD Vaccine produced in India

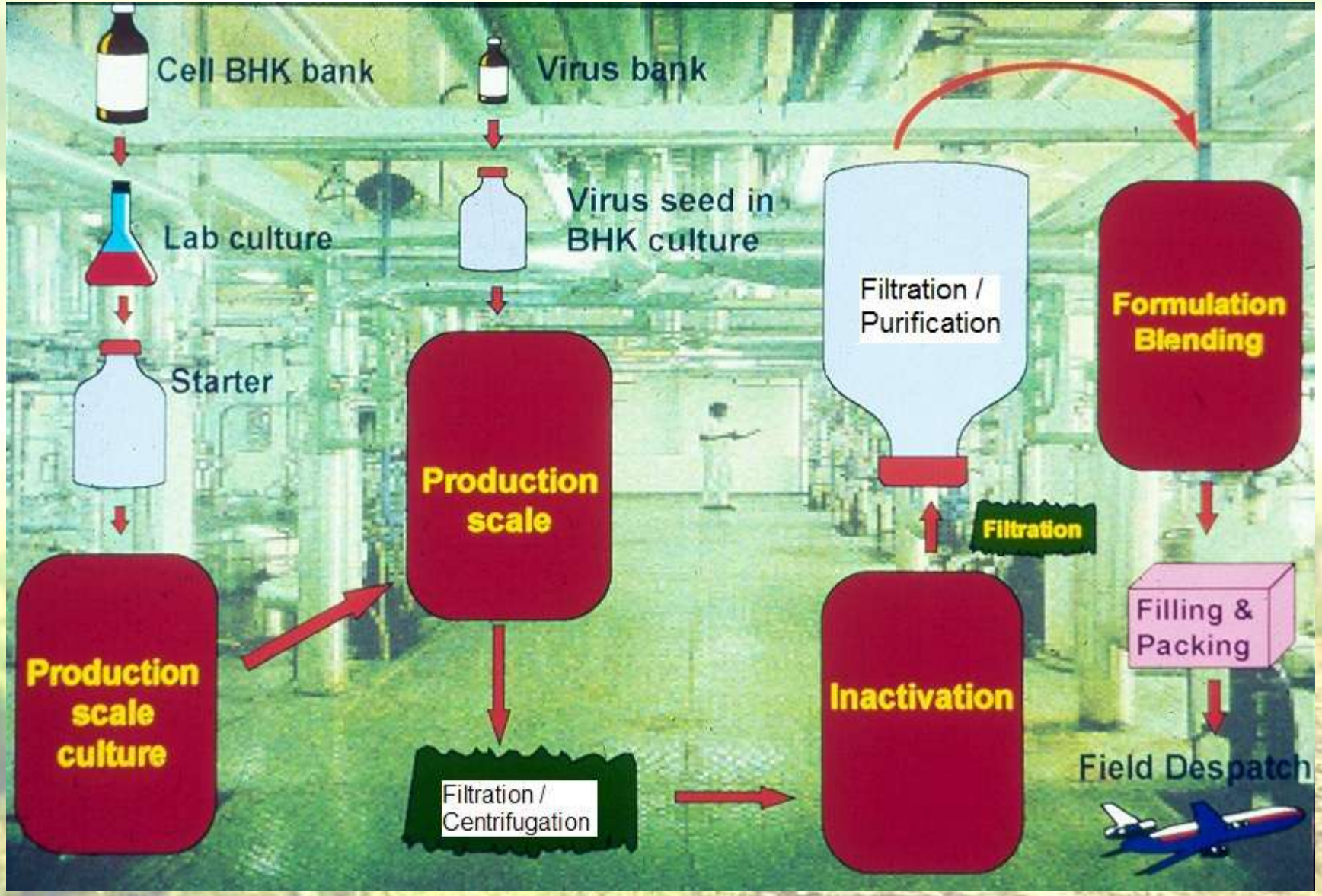
Mio. doses

Year	Quantity supplied		Total
	Institutions (FMDCP, ASCAD & Others	Trade	
2009-10	208.68	2.3	210.98
2010-11	183.62	2.1	185,72

Vaccine production technology

- All manufacturers use BHK suspension culture technique for manufacture of vaccine.
- Bioreactors/fermentors ranging from 50 L to 10000 L are being used for production of vaccine
- Binaryethyleneimine (BEI) is used as an inactivant.
- Aluminium hydroxide and oil adjuvant vaccines are manufactured.

Vaccine production flow diagram



Vaccine strain selection

- Until 2003 FMD vaccine manufacturers in India used different vaccine strains.
- Project Directorate on FMD (PDFMD), ICAR and Government of India harmonized the strains used for vaccine production.
- PDFMD undertakes responsibility of supplying the appropriate vaccine strain to FMD vaccine manufacturers.

Serotype	Vaccine strain	
Type O	O TNN 24/84 or O IND R2/75 until October 2003	O IND R2/75 from October 2003 onwards
Type A	A IND 17/82 or A IND 7/77 Till 2003	A IND 17/82 till September, 2008 A IND 40/00 from September 2008 onwards
Type Asia1	Asia1 WBN 117/85 or Asia1 IND 63/72 until October 2003	Asia1 IND 63/72 from October 2003 onwards
Type C	C IND 51/79 or C BOM 64 until October 2003	Discontinued since October 2003

Antigenic relationship between the field isolates (2001-2011) and the Bovine Vaccinate Serum of the (BVS) serotype O (O IND R2/75) vaccine strains

MER 57/2001	APV 94/2004	TNC 01/2006	IND506/08/AP	IND33/09/GJ
TNTn 62/2001	KAB 11/2004/2	APRr 20/2006	IND02/08/KL	ORKo 08/2010
GUK 142/2002	KAK 12/2004	TNKr 59/2007	IND158/08/KL	UPMe 11/2010
POP 144/2002	APKr 94/2005	TNKr 58/2007	IND323/08/MH	MAP 7/11/1
MAA 29/2002	APR 01/2005	KETr 24/2008	IND333/08/AS	
KETr 119/2002	KEEr 2/2005	KEI 18/2008	IND420/08/OR	
UPV 11/2003	KETr 83/2005	IND160/08/WB	IND287/09/UP	
ORC 20/2003	KET 89/2005/1	IND284/08/BR	APR 19/2009	
UPR 69/2003	MPSh 09/2005	IND290/08GJ	APR 23/2009	
KAK 40/2003	MAJ 90/2005/2	IND294/08/UP	CHRn 05/2009	
MAPb 37/03/5	PUL 88/2005	IND533/08/MP	GUK 26/2009	
RAJ 47/2003	TNTm 14/2005	IND157/08/GJ	KEKm 27/2009	
APKu 72/2004	APP 76/2005/2	IND554/08/WB	UPMe 10/2009	
APMb 76/2004	APRr 01/2005/3	IND320/08	GUK 29/2009	
APNa 08/2004/3	KAB 17/2005/3	IND09/08/TR	IND359/09/UP	

O IND R2/75

Combined data from IIL and Project Directorate on Foot and mouth Disease Annual report 2008-2011

Antigenic relationship between the field isolates (1968-2011) and the Bovine Vaccinate Serum of the (BVS) serotype A (A IND 40/00) vaccine strains

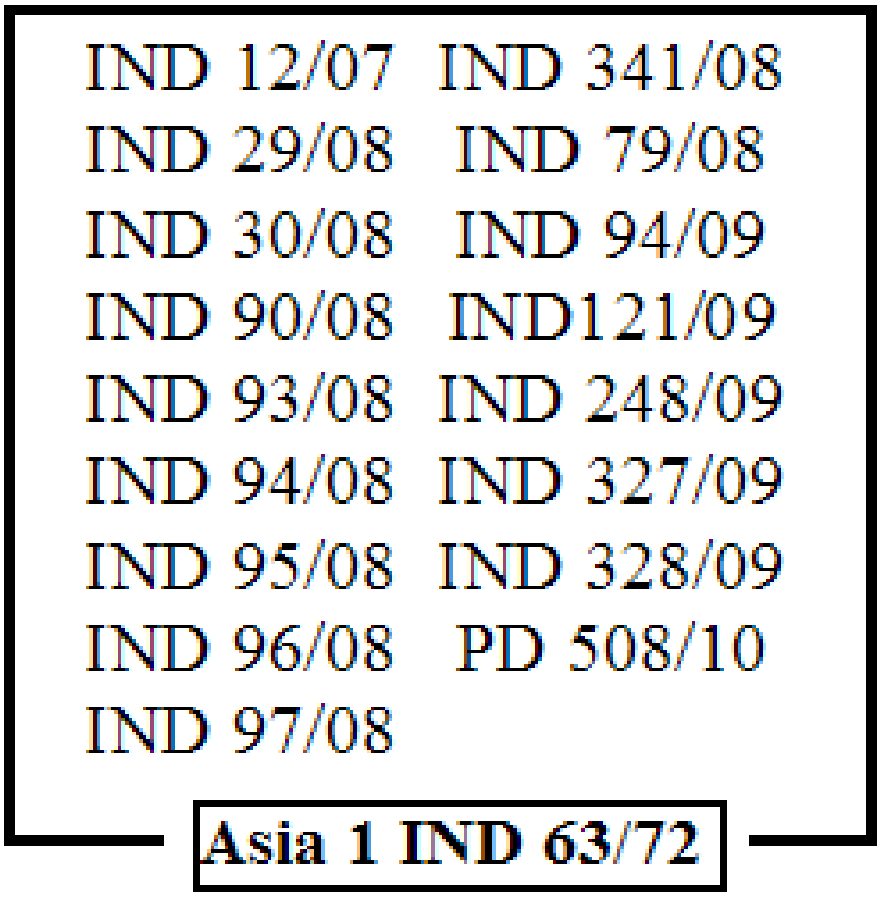
A IND 5/68	IND23/06	IND123/08	IND 749/09
IND17/77	IND106/06	IND314/08	IND 1/10
A ORS 66/84	IND 744/06	IND437/08	IND 46/10
A GUA 27/91	A KETr 04/07	IND 436/08	IND 61/10
A TNAn 60/94	A KETr 15/07	IND 438/08	IND 63/10
A HAH 14/00	A TNC 56/07/1	IND 09/09	IND 192/10
IND281/03	A TNK 57/07/1	IND 12/09	IND 194/10
WBJ 30/04	IND360/07	IND 13/09	IND 196/10
A KEW 20/05	IND417/07	IND 17/09	IND 747/10
A APS 66/05	IND407/07	IND 332/09	IND 226/10
IND101/06	IND245/07	IND 333/09	IND 197/10
IND28/06	IND195/07	IND 747/09	TNS 4/11/3

A IND 40/00

IND17/82	IND97/06	IND 17/09
A RAJ 21/96	IND53/08	IND 45/10
IND461/02	IND17/09	IND 136/10

Combined data from IIL and Project Directorate on Foot and mouth Disease Annual report 2008-2011

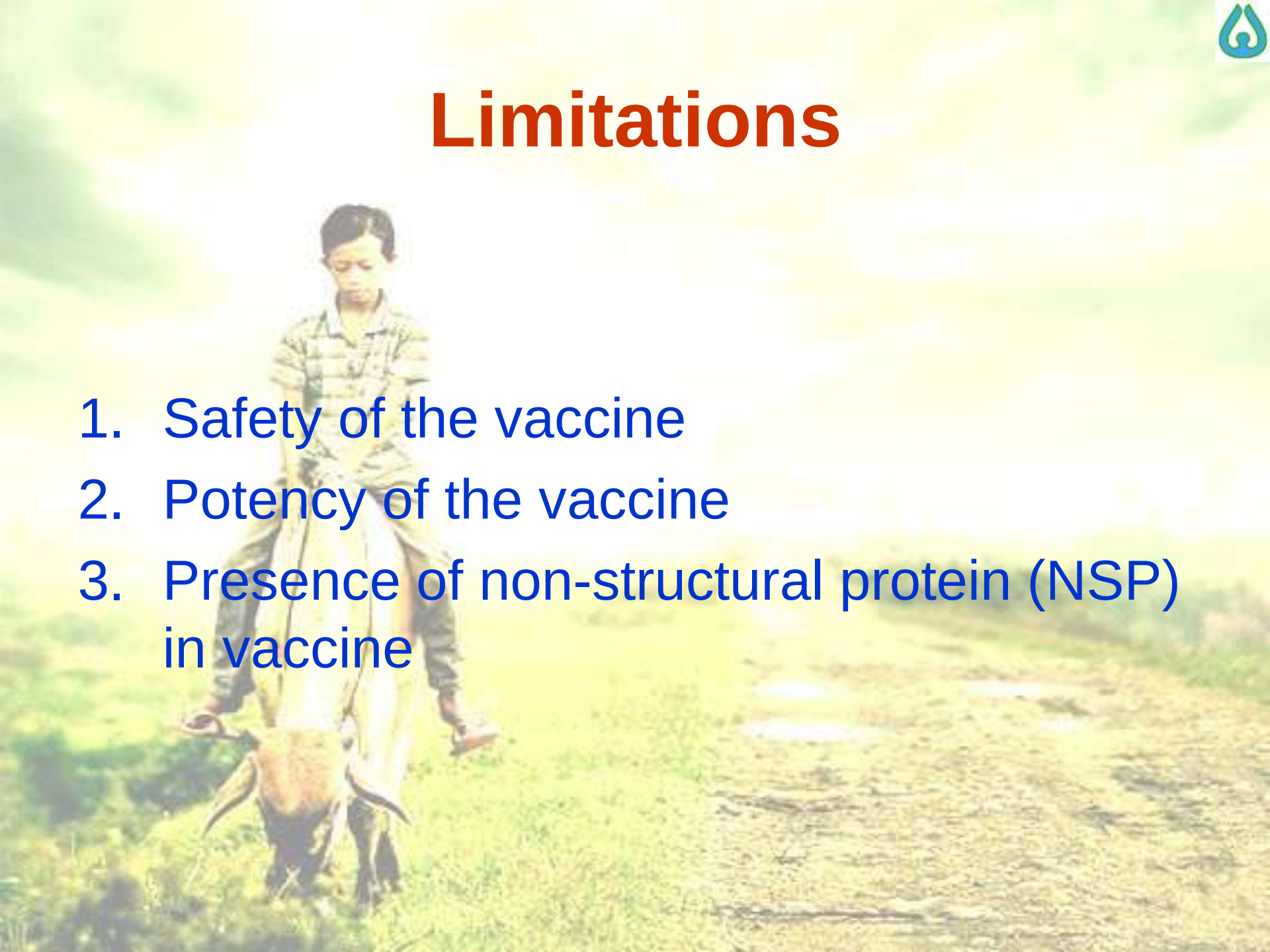
Antigenic relationship between the recent isolates (2007-2010) and the Bovine Vaccinate Serum of the (BVS) serotype Asia 1 (Asia 1 IND 63/72) vaccine strains



Adapted from Project Directorate on Foot and mouth Disease Annual report 2008-2011

2. Limitations in the inactivated FMD vaccine

Limitations



1. Safety of the vaccine
2. Potency of the vaccine
3. Presence of non-structural protein (NSP) in vaccine

Limitations

- Safety of the vaccine
  - Does the vaccine incorporates completely inactivated antigen?
    - Inactivation kinetics
    - Amplification test
    - Innocuity / safety test in animals

Inactivation of FMD virus

Average inactivation kinetics of two lots of FMD virus serotypes O, A, and Asia-1 inactivated with five different concentrations of BEI

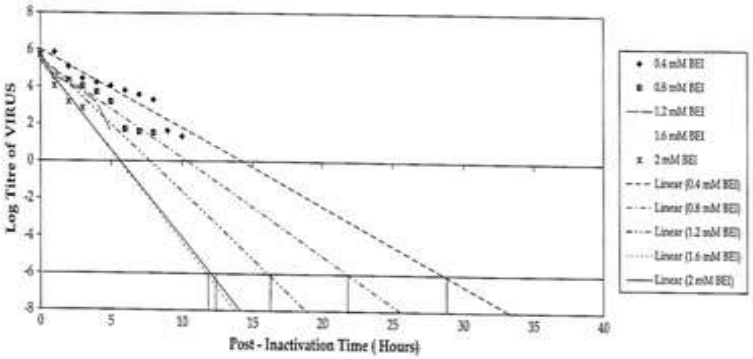
Kinetics	0.4 mM			0.8 mM			1.2 mM			1.6 mM			2.0 mM		
	O	A	Asia-1	O	A	Asia-1	O	A	Asia-1	O	A	Asia-1	O	A	Asia-
$t_{1/2}$ (min)	44.778	40.772	36.181	31.84	30.632	31.34	20.95	18.226	22.822	16.835	17.418	14.59	14.278	12.569	10.8
SD$t_{1/2}$ (min)	4.482	7.803	4.082	2.537	3.628	2.718	2.502	4.609	2.095	1.42	3.045	1.186	2.49	2.139	0.3
M	−0.403	−0.443	−0.499	−0.567	−0.59	−0.576	−0.862	−0.991	−0.791	−1.073	−1.037	−1.238	−1.265	−1.437	−1.6
SDM	0.04	0.085	0.056	0.045	0.07	0.05	0.103	0.251	0.073	0.091	0.181	0.101	0.221	0.245	0.0
HRS	29.483	24.035	22.639	20.432	18.083	19.161	13.766	10.882	13.953	10.862	10.326	8.973	9.243	7.424	6.5

$t_{1/2}$ = half-life of the virus; SD$t_{1/2}$ = standard deviation of the half-life; *M* = slope; SDM = standard deviation of the slope; HRS = hours requir for complete inactivation.

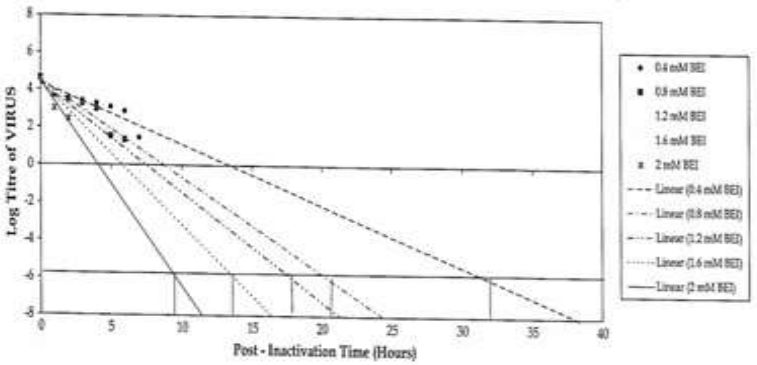
Aarti et al., 2004 Biologicals

Inactivation of virus

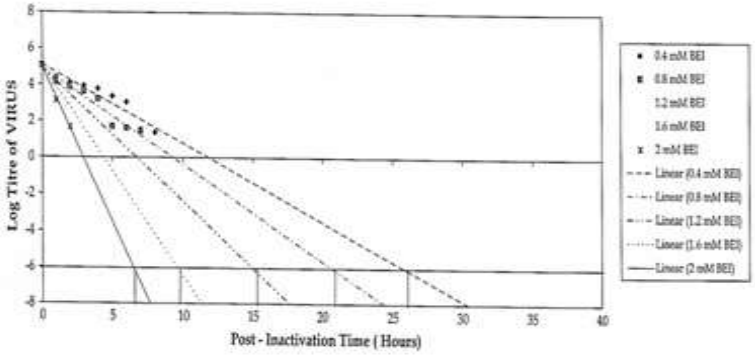
- Inactivation
  - Validation
    - Validation of binary ethyleneimine (BEI) used as an inactivant for foot and mouth disease tissue culture vaccine. Aarthi D., Rao K.A., Robinson R., Srinivasan V.A. (2004). *Biologicals*. **32**, 153-156.



Trend analysis of FMD virus (type O IND R 2/75) inactivation with different concentrations of BEI.



Trend analysis of FMD virus (type A IND 17/82) inactivation with different concentrations of BEI.



Trend analysis of FMD virus (type Asia-1 IND 63/72) inactivation with different concentrations of BEI.

Limitations

- Potency of FMD vaccine
  1. Does the existing potency adequate for the use in control programmes?
  2. Vaccine potency test - Should this be left to the vaccine manufacturer?

Limitations

- FMD vaccine Potency
  - Current requirements - ≥ 3 PD_{50}
    - European Pharmacopoeia - 2010
    - British Pharmacopoeia 2010
    - Indian Pharmacopoeia 2010

Limitations

- FMD vaccine potency
  - Is it necessary to increase the potency values from ≥ 3 PD_{50} to ≥ 6 PD_{50} ?
  - It will be ideal to fix the potency value at ≥ 6 PD_{50}

Limitations

- FMD vaccine potency
  - Current trend – all vaccine manufacturers are responsible for assessing the potency.
  - It is ideal to establish an independent FMD vaccine testing laboratory where all the parameters of vaccine including the potency will be assessed.
  - Government of India / ICAR shall consider this aspect seriously.

Limitations

- FMD Vaccine free of NSP
  - How important is it to produce FMD vaccine free of NSP?
  - Current status – vaccine manufactured in India varies from NSP free to traces of NSP.
  - No separate claims on absence of NSP is being made

Limitations

- FMD Vaccine free of NSP
  - The usefulness of NSP free vaccine in endemic countries is limited.
  - Useful in countries free of FMD with vaccination
  - Useful in areas which are undertaking mass vaccinations – to detect the incidence of disease in vaccinated animals

Current method of testing vaccine for NSP

- OIE protocol

Limitations

Limitations of the NSP antibody test

1. Needs disease / antibody free animals for testing.
2. Test takes 90 days for completion. Release of vaccine is likely to be delayed.
3. Vaccine batch will be rejected if the test fails

NSP detection in antigen lots

Advantages:

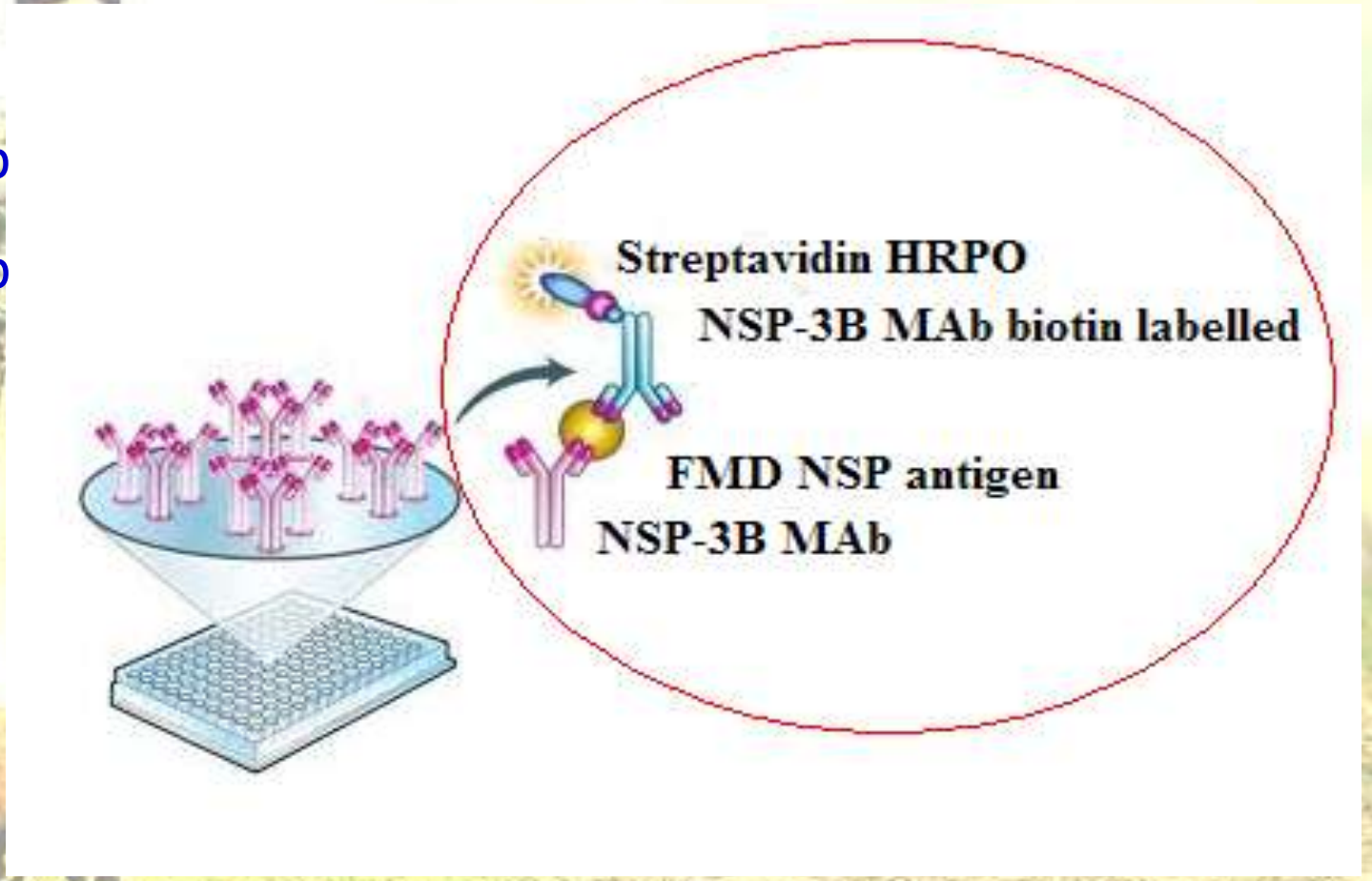
1. Each lot of antigen can be tested.
2. Can be used as an in-process control test.
3. Passed antigen lots can alone be incorporated in the vaccine.
4. Animal tests can be avoided
5. Saves time

NSP antigen quantification ELISA format

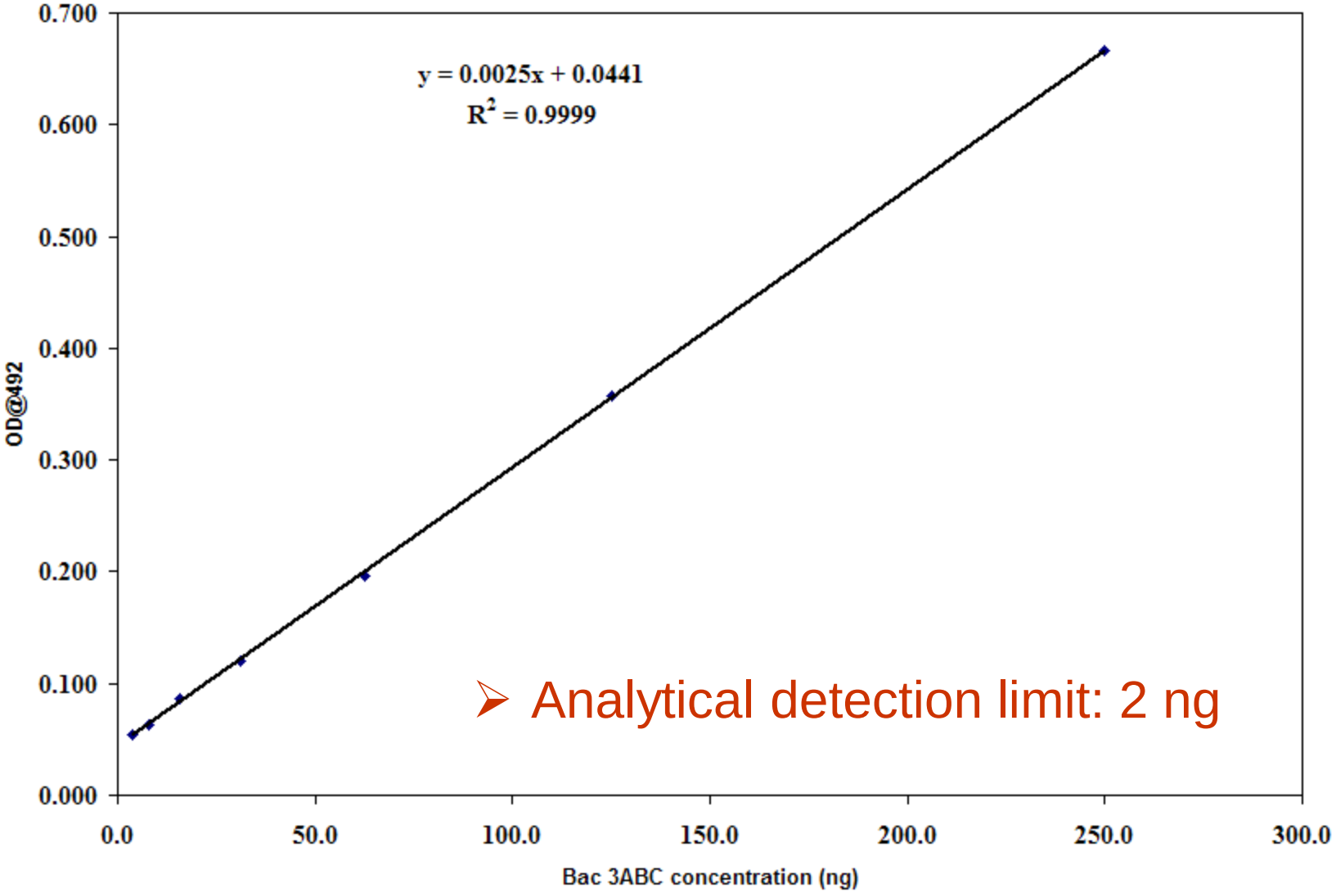
Baculovirus expressed FMD NSP mutated 3ABC antigen

FMD NSP 3B MAb

FMD NSP 3B MAb biotin labeled



Standard curve



- Analytical detection limit: 2 ng

FMD NSP quantification

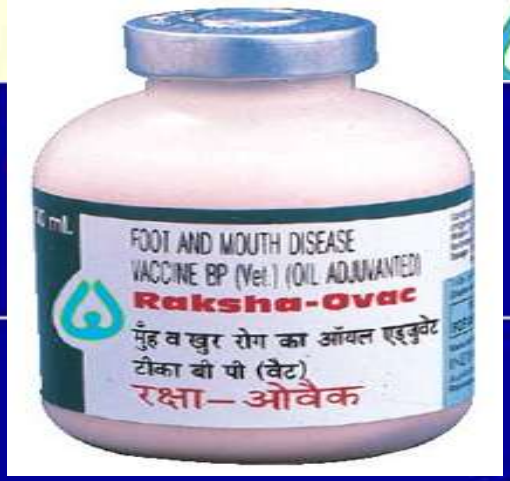
Samples	FMD NSP concentration (ng/ml)		
	O	A	Asia 1
24 h post infection	500.12	245.83	315.91
40 h post infection	498.97	256.50	100.23
Post clarification	432.31	183.50	101.14
24 h post inactivation	359.15	162.83	106.36
48 h post inactivation	352.31	150.00	118.30
Concentrate	570.77	281.17	195.23

Tests to decide the quantity of NSP antigen in the vaccine

- To find out minimum FMD NSP antigen elicits NSP immune response in cattle
- Animal experiments using recombinant FMD NSP antigen and FMD vaccine spiked with native FMD NSP antigen.

3. FMD vaccine – Cold chain

The journey of a product



Mfg Site

FEW DAYS

Warehouse

1-5 MONTHS

Depots

1-3 MONTHS

Wholesaler

1-2 MONTHS

Pharmacy

0.5-1 MONT

Patient

Drug company's ownership

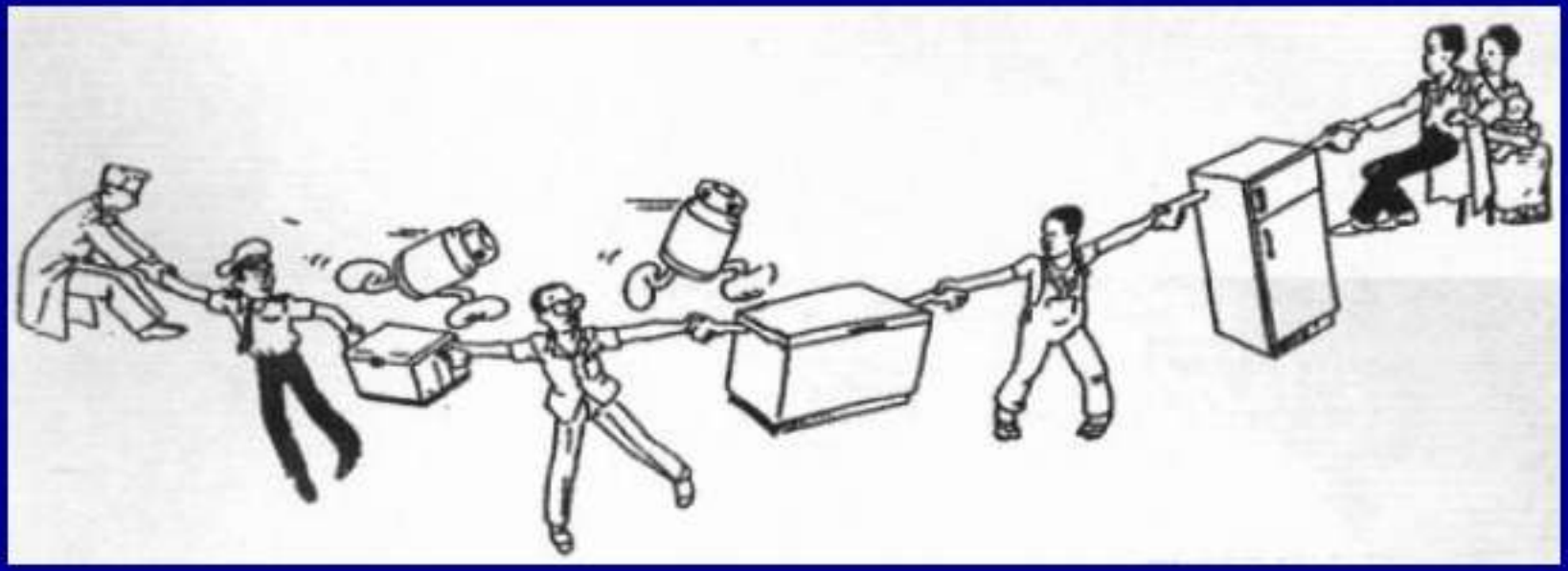
Distributors ownership

Cold Chain Maintenance - Challenge

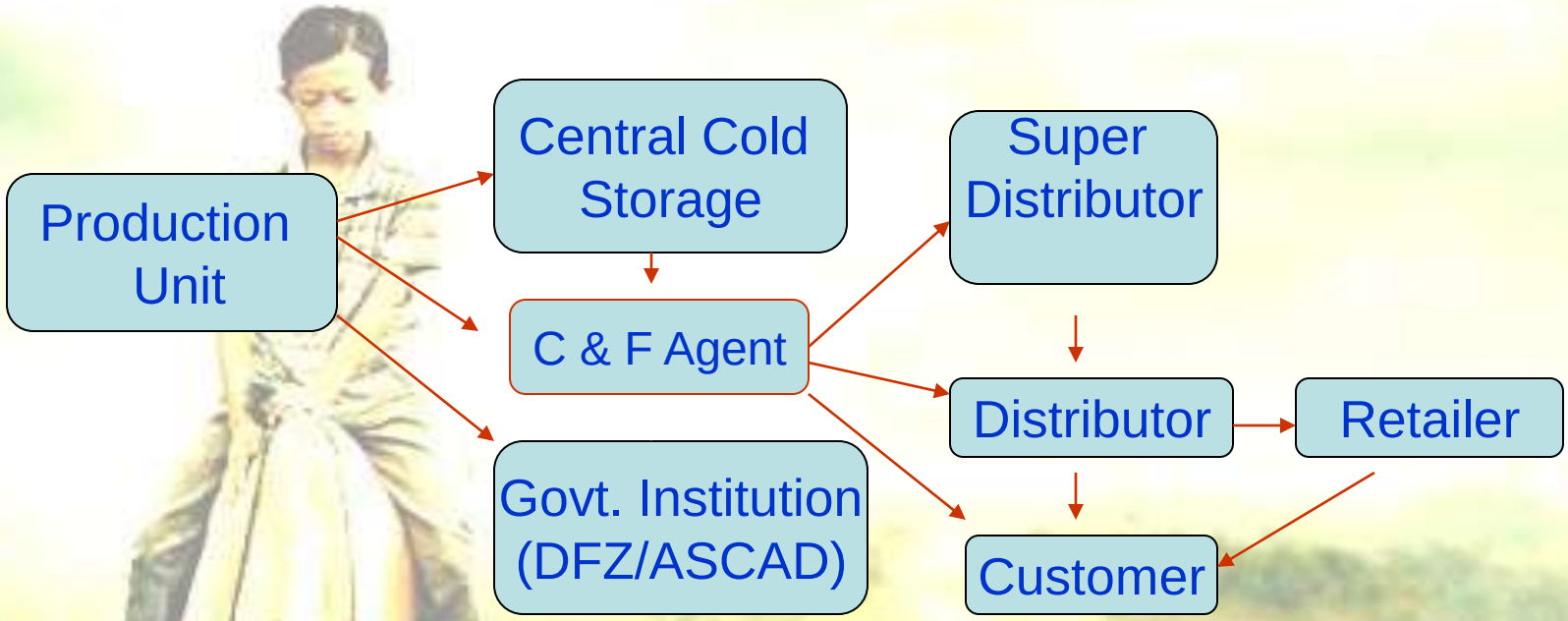
The Challenge

Is the patient receiving the right drug ?
and in right condition

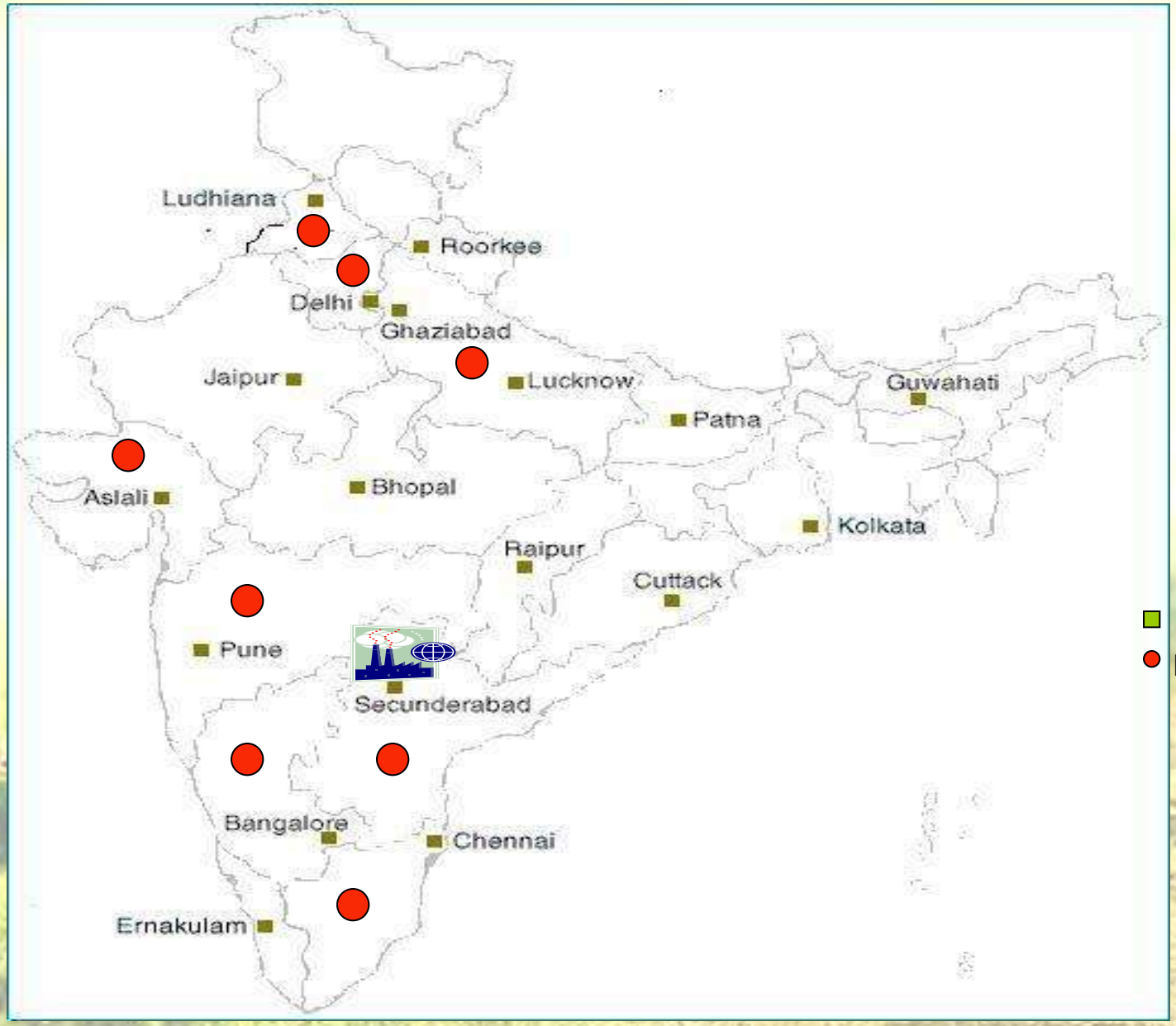
?Who owns the chain ?



Vaccine Movement – Distribution Channel



IIL Logistics Network



Mode of Transportation

The new depot at Kwai Fong will help TNT meet surging demand for the transhipment of goods in and out of China

Messenger Service

4. FMD Control programme

FMD control programme

1. Government of India programme – FMD control programme
2. Assistance to the State Governments for control of Animal Diseases (ASCAD)
3. Dairy cooperatives
4. Trade

Government of India - FMDCP

- 2003 to date 54 districts in the following States are being covered under this programme.

State	no of districts
Kerala	3
Tamilnadu	1
Andhra Pradesh	4
Maharashtra	6
Gujarat	4
Harayana	10
Punjab	10
Uttar Pradesh	16
Total	**54**
Union territories	
Delhi	Total area
Andhaman & Nicobar	Total area
Lakshadweep	Total area

FMDCP

- 2010 – 2011 – continuing
- States Districts

Kerala	14
Tamilnadu	30
Andhra Pradesh	23
Karnataka	30
Maharashtra	36
Gujarat	26
Haryana	20
Punjab	17
Uttar Pradesh	16
Total	**212 (221)**

- All the districts in the above States will be covered.
- All the union territories & Goa are covered


FMD Control Program
Govt. of India
JAMMU & KASHMIR
Srinagar
HIMACHAL PRADESH
Shimla
PAKISTAN
CHINA (TIBET)
PUNJAB
UTTARANCHAL
Dehradun
DELHI
HARYANA
ARUNACHAL PRADESH
SIKKIM
NEPAL
UTTAR PRADESH
BHUTAN
Itanagar
RAJASTHAN
Jaipur
Lucknow
Patna
BIHAR
ASSAM
Dispur
NAGALAND
Kohima
Shillong
Imphal
MANIPUR
MEGHALAYA
Agartala
TRIPURA
Aizawl
MIZORAM
GUJARAT
Gandhinagar
Bhopal
MADHYA PRADESH
Ranchi
JHARKHAND
WEST BENGAL
Calcutta
MYANMAR
CHHATTISGARH
Raipur
ORISSA
Bhubaneshwar
BANGLADESH
DIU
DAMAN
DADAR & NAGAR HAVELI
MAHARASHTRA
Mumbai (Bombay)
ARABIAN SEA
Hyderabad
ANDHRA PRADESH
YANAM (Pondicherry)
BAY OF BENGAL
Panaji
GOA
KARNATAKA
Bangalore
LAKSHADWEEP
Chennai (Madras)
PONDICHERRY (Puduchchery)
MAHE (Pondicherry)
TAMILNADU
KARAIKAL (Pondicherry)
Port Blair
KERALA
ANDAMAN & NICOBAR ISLANDS
Thiruvananthapuram
N
Map not to Scale


FMD Control Program - Southern Peninsula
JAMMU & KASHMIR
Srinagar
HIMACHAL PRADESH
Shimla
PAKISTAN
Chandigarh
PUNJAB
UTTARANCHAL
Dehradun
DELHI
HARYANA
ARUNACHAL PRADESH
SIKKIM
NEPAL
UTTAR PRADESH
Gangtok
BHUTAN
Itanagar
RAJASTHAN
Jaipur
Lucknow
Patna
BIHAR
ASSAM
Dispur
NAGALAND
Kohima
Shillong
Imphal
MANIPUR
MEGHALAYA
Agartala
TRIPURA
Aizawl
MIZORAM
GUJARAT
Gandhinagar
Bhopal
MADHYA PRADESH
Ranchi
JHARKHAND
WEST BENGAL
Calcutta
MYANMAR
CHHATTISGARH
Raipur
ORISSA
Bhubaneshwar
BANGLADESH
DIU
DAMAN
DADAR & NAGAR HAVELI
MAHARASHTRA
Mumbai (Bombay)
ARABIAN SEA
Hyderabad
ANDHRA PRADESH
YANAM (Pondicherry)
BAY OF BENGAL
Panaji
GOA
KARNATAKA
Bangalore
Chennai (Madras)
PONDICHERRY (Puduchchery)
LAKSHADWEEP
MAHE (Pondicherry)
TAMILNADU
KARAIKAL (Pondicherry)
Port Blair
ANDAMAN & NICOBAR ISLANDS
KERALA
Thiruvananthapuram
Map not to Scale

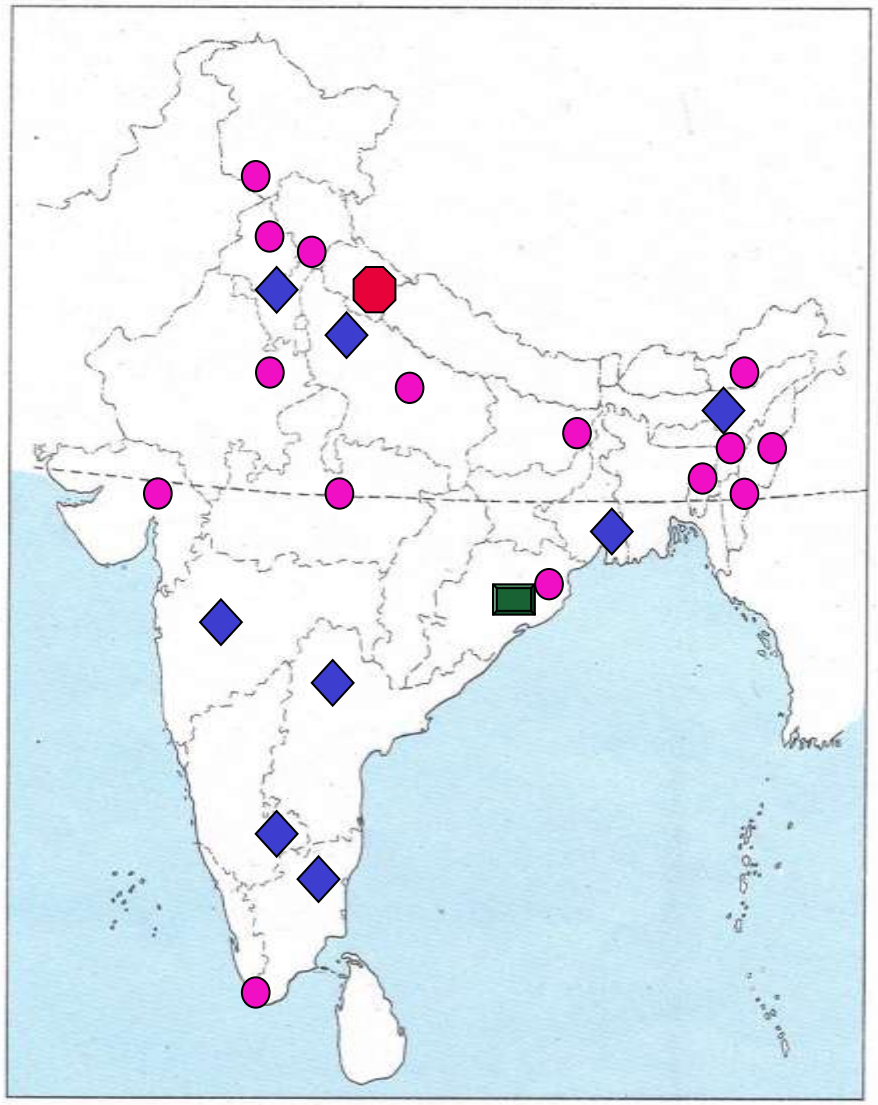
FMD control programme

- Surveillance
  - Currently post-marketing surveillance is left to the manufacturers.
  - Ideally an independent agency should be entrusted with the responsibility of conducting FMD vaccine post-marketing surveillance.

FMD control programme

- Surveillance in FMD control project area
  - State Governments collect serum samples from vaccinated animals in FMDCP area.
  - The serum samples are analyzed by the PDFMD laboratories located in different States.
  - The State Governments and PDFMD regularly undertakes disease surveillance through the epidemiological units.

Project Directorate on FMD and Epidemiological Units



- Central FMD Laboratory – PD FMD
- Regional Centers (8)
- Network Units (15)
- SAARC FMD Centre

Percentage of animals showing antibody titers of ≥1.8 log10 against FMD virus – 30 DPV

Phase	Type O		Type A		Type Asia 1	
	Pre-Vaccination	Post-Vaccination	Pre-Vaccination	Post-Vaccination	Pre-Vaccination	Post-Vaccination
IX	63.7	85.6	52	73.3	52.6	73
X	63.4	87.4	50.6	74.1	48.9	76.7

Source: PD FMD Annual report 2010-11

Recommendations for consideration

1. Vaccine manufacturers may undertake validation of inactivation kinetics while introducing new batches of BEA/BEI.
2. Potency of FMD vaccine to be increased to 6 PD_{50} (?)
3. An independent agency to carry out FMD vaccine batch testing and provide the manufacturers with the batch release certificate.
4. Post-marketing surveillance should be made mandatory.

Acknowledgements

IIL

M. MADHANMOHAN

B. MOHAPATRA

NSN BHARGAV

RAMNATH KAVLE

PDFMD

B.PATTNAIK

A. SANYAL

J. K. MOHAPATRA

Thank you