

# Progressive Control of Foot and Mouth Disease in Pakistan

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**Progressive Control of FMD in  
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# Livestock Population

## Large ruminants

| Species      | Population<br>( in millions) |
|--------------|------------------------------|
| Cattle       | 36.9                         |
| Buffaloes    | 32.7                         |
| <b>Total</b> | <b>69.6</b>                  |

## Small ruminants

| Species      | Population<br>( in millions) |
|--------------|------------------------------|
| Sheep        | 28.4                         |
| Goats        | 63.1                         |
| <b>Total</b> | <b>91.5</b>                  |

**Total ruminants population = 161.1 millions**

# Livestock Production Systems

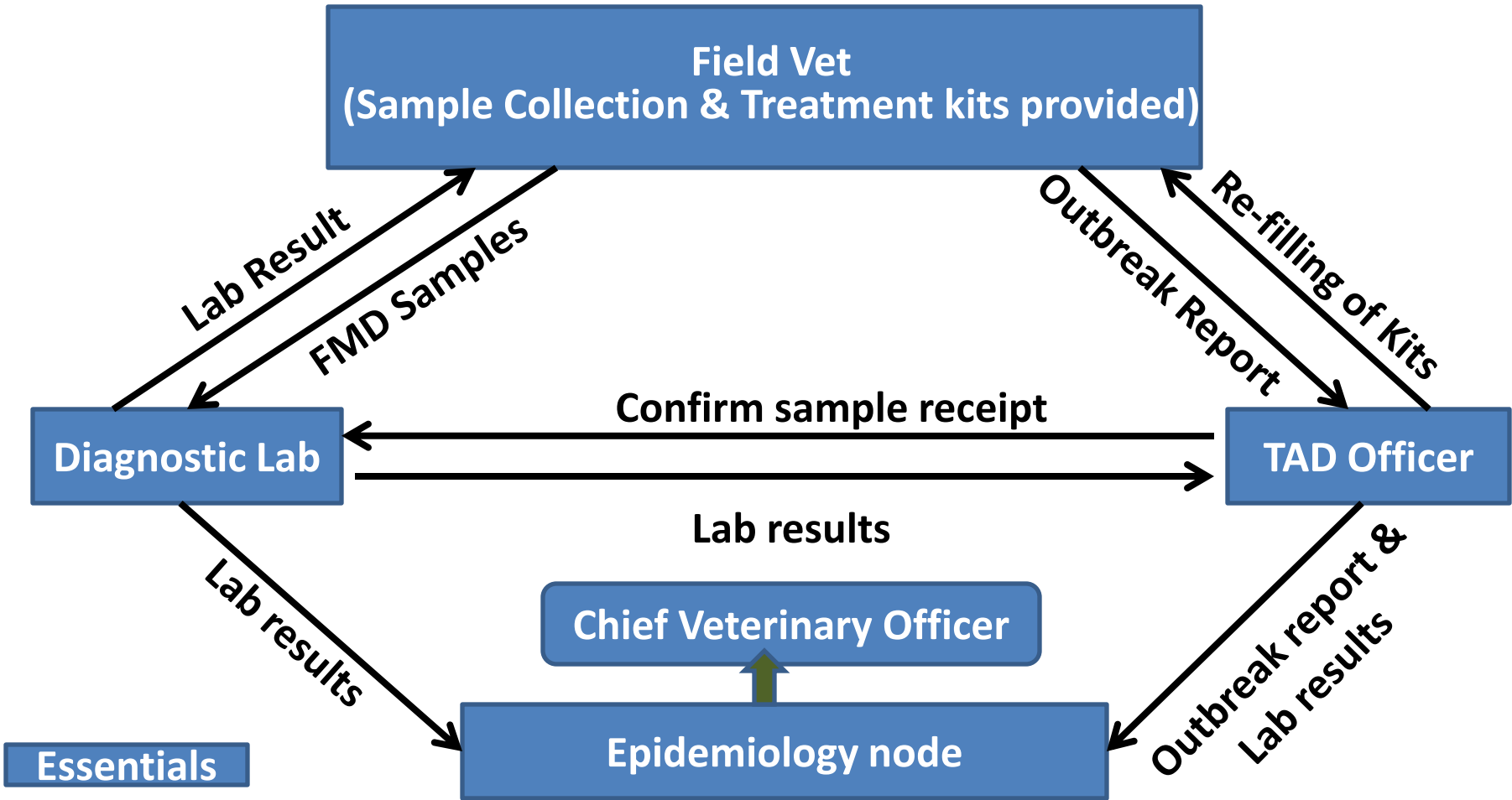
- **Large Ruminants**

- Subsistence small holdings
- Small market oriented small holdings
- Rural commercial farms
- Urban/peri urban dairy farming (dairy colonies)
- Desert cattle farming

- **Small Ruminants**

- Nomadic
- Transhumant
- Stationary

# Surveillance Model for FMD

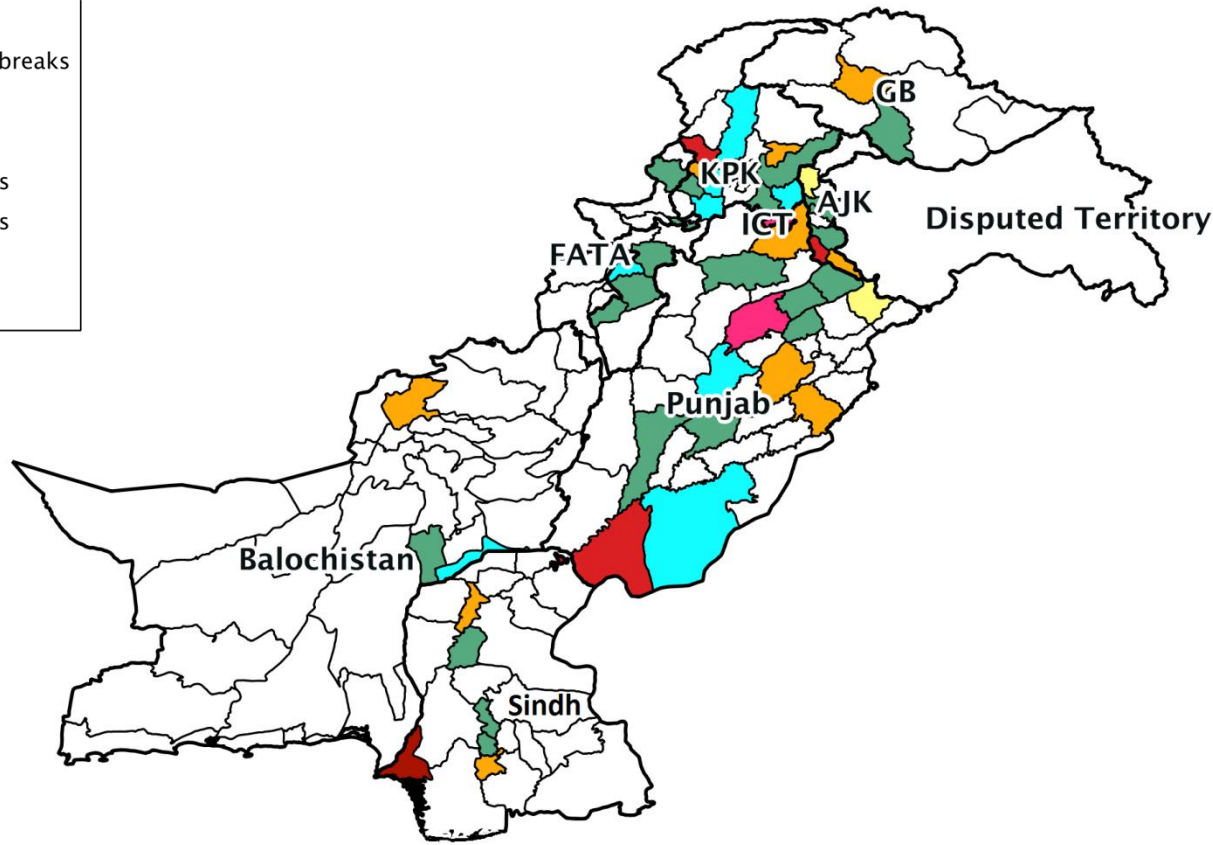
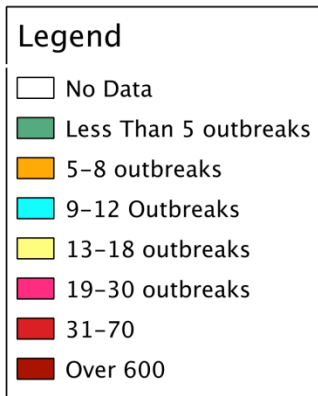


|  |   |
|--|---|
| Awareness of field staff and dairy farmers           | Training of veterinary staff                        |
| Provision of sample collection and dispatch material | Cover expenditure of sample collection and dispatch |
| Respond back with outbreak handling                  | Report back to field staff on lab findings          |

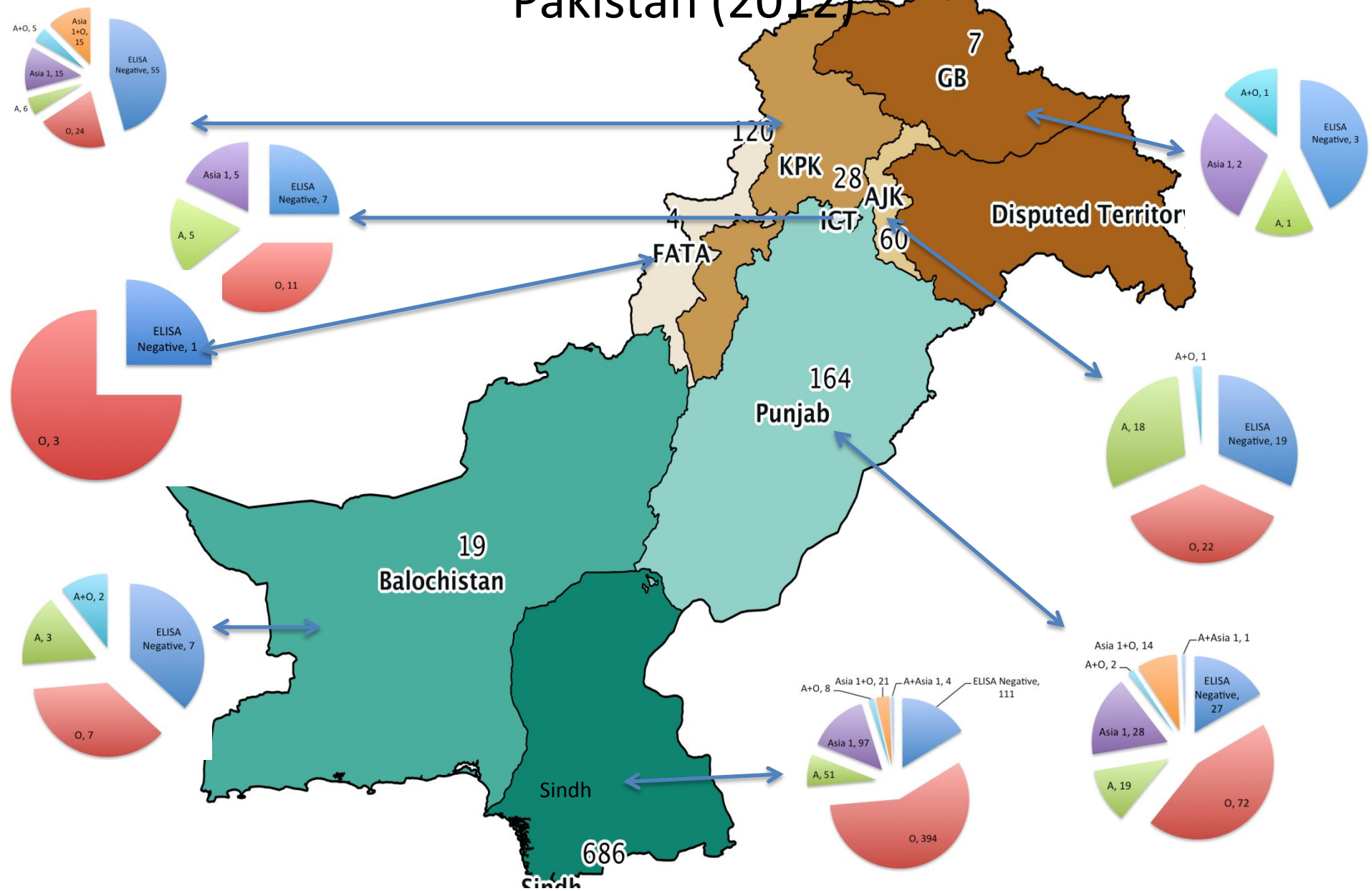
## FMD Virus Serotypes (Jan - Dec 2012)

| Province           | Total FMD outbreaks | Outbreak (#) due to serotype |            |            |           |           | A+ Asia-1 | ELISA Neg Outbreaks |
|--------------------|---------------------|------------------------------|------------|------------|-----------|-----------|-----------|---------------------|
|                    |                     | O                            | A          | Asia-I     | O+ Asia-I | O+A       |           |                     |
| Punjab             | 164                 | 72                           | 19         | 28         | 14        | 2         | 1         | 27(+1)              |
| Sindh              | 686                 | 394                          | 51         | 97         | 21        | 8         | 4         | 111                 |
| Khyber Pakhtunkhwa | 120                 | 24                           | 6          | 15         | 15        | 5         | -         | 55                  |
| Balochistan        | 19                  | 7                            | 3          | -          | -         | 2         | -         | 7                   |
| Gilgit-Baltistan   | 7                   | -                            | 1          | 2          | -         | 1         | -         | 3                   |
| FATA               | 4                   | 3                            | -          | -          | -         | -         | -         | 1                   |
| AJK                | 60                  | 22                           | 18         | -          | -         | 1         | -         | 19                  |
| Islamabad          | 28                  | 11                           | 5          | 5          | -         | -         | -         | 7                   |
| <b>Total</b>       | <b>1088</b>         | <b>533</b>                   | <b>103</b> | <b>147</b> | <b>50</b> | <b>19</b> | <b>5</b>  | <b>230(+1)</b>      |

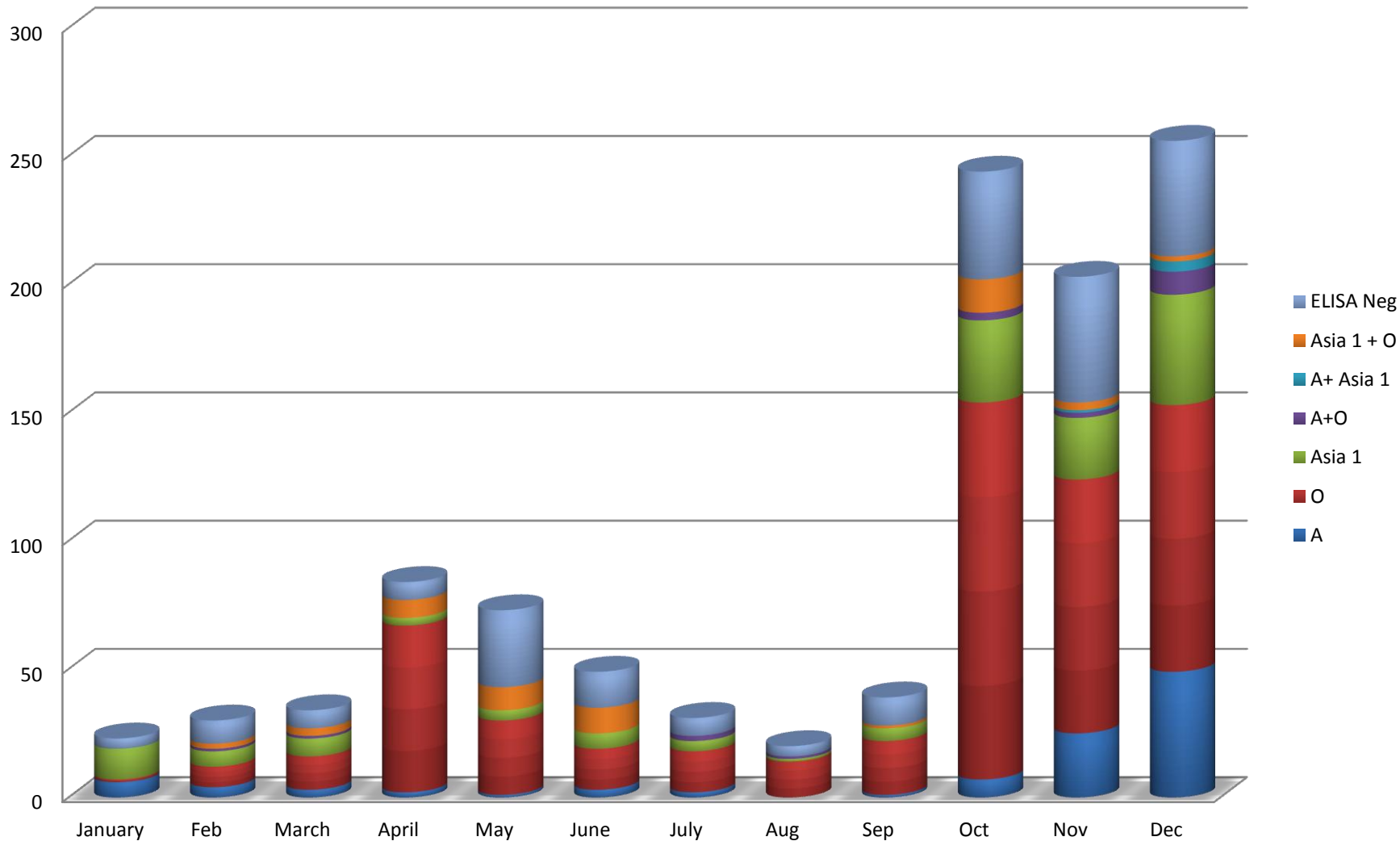
# District level distribution of FMD outbreaks in Pakistan (2012)



# Regional/Provincial Distribution of FMD outbreaks in Pakistan (2012)



# Monthly Distribution of FMDV serotypes in Pakistan (2012)



## FMD Virus Serotypes (Jan - March 2013)

| Province              | Total FMD outbreaks | Outbreak (#) due to serotype |            |           |              |          |              | ELISA Neg Outbreaks |
|-----------------------|---------------------|------------------------------|------------|-----------|--------------|----------|--------------|---------------------|
|                       |                     | O                            | A          | Asia-I    | O+<br>Asia-I | O+A      | A+<br>Asia-1 |                     |
| Punjab                | 53                  | 14                           | 17         | 10        | -            | 1        | -            | 11                  |
| Sindh                 | 371                 | 152                          | 132        | 28        | 3            | 5        | 2            | 44(5)               |
| Khyber<br>Pakhtunkhwa | 38                  | 7                            | 5          | 2         | 6            | 8        | -            | 10                  |
| Balochistan           | 5                   | 2                            | -          | -         | -            | -        | -            | 1(2)                |
| Gilgit-<br>Baltistan  | -                   | -                            | -          | -         | -            | -        | -            | -                   |
| FATA                  | 1                   | -                            | -          | -         | -            | -        | -            | 1                   |
| AJK                   | 41                  | 1                            | 13         | 4         | -            | -        | -            | 10                  |
| Islamabad             | 3                   | -                            | -          | 2         | -            | -        | -            | (1)                 |
| <b>Total</b>          | <b>512</b>          | <b>176</b>                   | <b>167</b> | <b>46</b> | <b>14</b>    | <b>9</b> | <b>2</b>     | <b>77(21)</b>       |

# Characterization of Pakistani FMDV Isolates (WRL, Pirbright)

- Number of samples = 10+35 in two batches
- **Genotyping**
  - Batch 1: A-Iran05<sup>HER-10</sup> (3 isolates); Asia-I (SINDH-08) /Group-VII (7 isolates)
  - Batch 2: 35 samples sent on 13/12/2012; Serotyping results received on 5/2/2013, Genotyping results shown in next slides
- **Vaccine Matching**
  - Batch 1:
    - a) A/PAK/6/2012 matched well with A22/IRQ and A/TUR/06 but failed to match with A/Iran05
    - b) Asia-1 viruses (As/PAK/91/2011 and As/PAK/5/2012 failed to match with As/IND/8/79. However, one of the two matched with As/Shamir
  - Batch 2:
    - a) 2/3 serotype A viruses matched with A Tur 06 and A22 Irq, one matched with A May 97 and Iran 05 and no matching with Indian 17/82
    - b) Vaccine matching (O, Asia-1) results still awaited

# Report on FMDV O in Pakistan in 2012

Batch: WRLFMD/2012/00037

◆ indicates viruses in this batch

Software: MEGA 5.1

Analysis

Analysis ----- Phylogeny Reconstruction

Scope ----- All Selected Taxa

Statistical Method ----- Neighbor-joining

Phylogeny Test

Test of Phylogeny ----- Bootstrap method

No. of Bootstrap Replications ----- 1000

Substitution Model

Substitutions Type ----- Nucleotide

Model/Method ----- Kimura 2-parameter model

Substitutions to Include ----- d: Transitions + Transversions

Rates and Patterns

Rates among Sites ----- Uniform rates

Pattern among Lineages ----- Same (Homogeneous)

Data Subset to Use

Gaps/Missing Data Treatment ----- Pairwise deletion

Codons Included ----- 1st+2nd+3rd+Non-Coding

No. of Sites : 642

No Of Bootstrap Reps = 1000

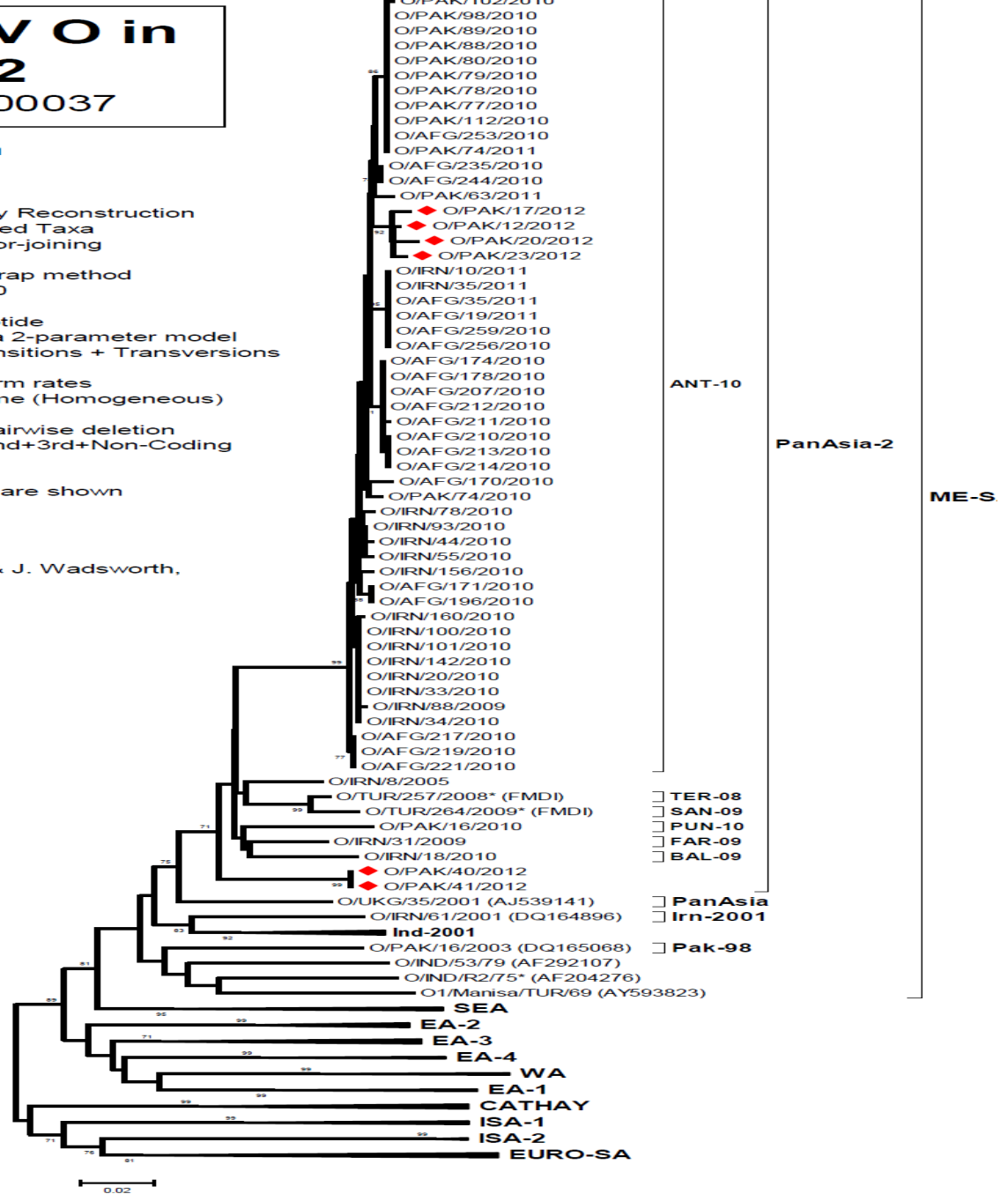
Only bootstrap values of 70% and above are shown

\*, not a WRLFMD Ref. No.

N.J. Knowles, K. Bachanek-Bankowska & J. Wadsworth,  
26 February 2013

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# Report on FMDV A in Pakistan in 2012

Batch: WRLFMD/2012/00037

◆ indicates viruses in this batch

Software: MEGA 5.1

Analysis

Analysis ————— Phylogeny Reconstruction

Scope ————— All Selected Taxa

Statistical Method ————— Neighbor-joining

Phylogeny Test

Test of Phylogeny ————— Bootstrap method

No. of Bootstrap Replications — 1000

Substitution Model

Substitutions Type ————— Nucleotide

Model/Method ————— Kimura 2-parameter model

Substitutions to Include ————— d: Transitions + Transversions

Rates and Patterns

Rates among Sites ————— Uniform rates

Pattern among Lineages ————— Same (Homogeneous)

Data Subset to Use

Gaps/Missing Data Treatment ————— Pairwise deletion

Codons Included ————— 1st+2nd+3rd+Non-Coding

No. of Sites : 639

No Of Bootstrap Reps = 1000

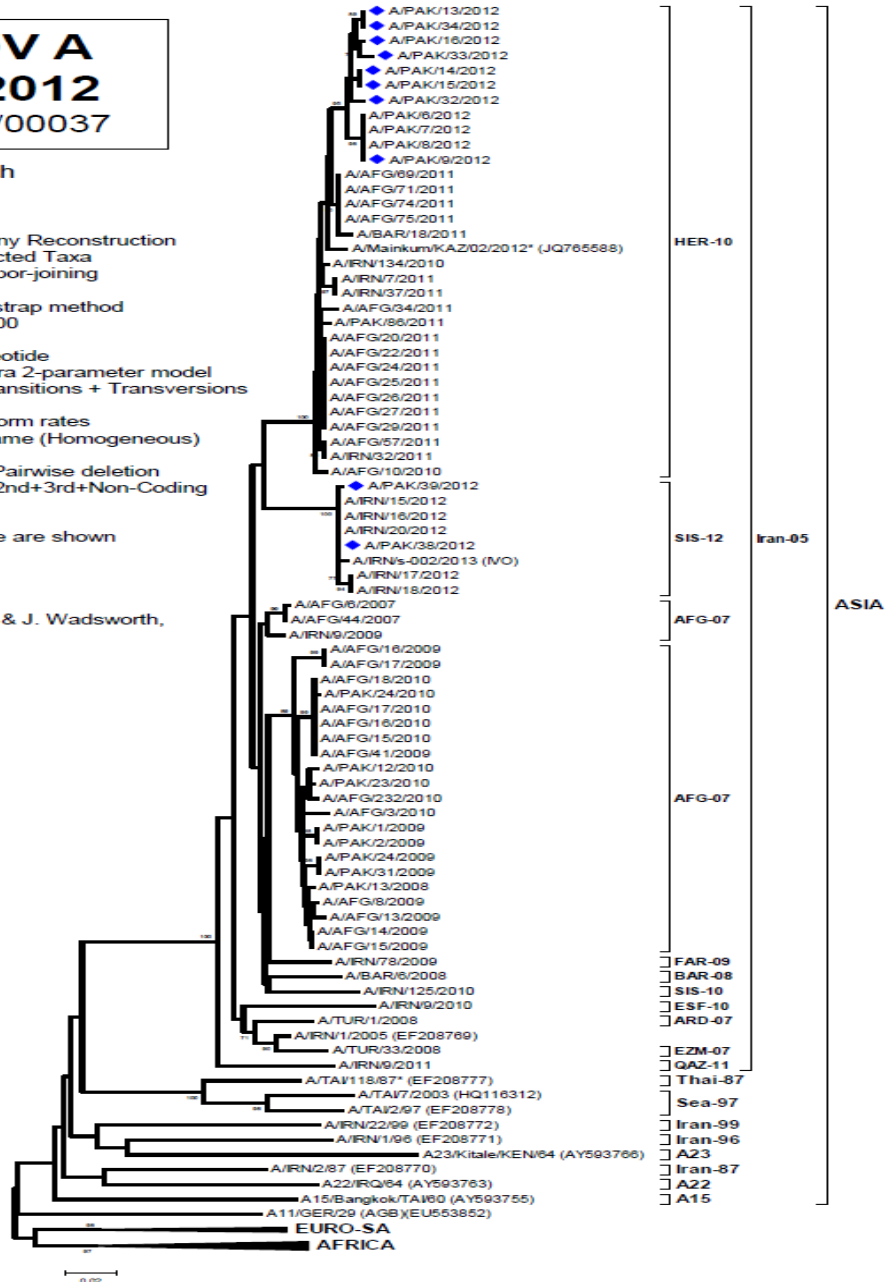
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# Report on FMDV Asia 1 in Pakistan in 2012

Batch: WRLFMD/2012/00037

◆ indicates viruses in this batch

Software: MEGA 5.1

Analysis

Analysis \_\_\_\_\_ Phylogeny Reconstruction

Scope \_\_\_\_\_ All Selected Taxa

Statistical Method \_\_\_\_\_ Neighbor-joining

Phylogeny Test

Test of Phylogeny \_\_\_\_\_ Bootstrap method

No. of Bootstrap Replications \_\_\_\_\_ 1000

Substitution Model

Substitutions Type \_\_\_\_\_ Nucleotide

Model/Method \_\_\_\_\_ Kimura 2-parameter model

Substitutions to Include \_\_\_\_\_ d: Transitions + Transversions

Rates and Patterns

Rates among Sites \_\_\_\_\_ Uniform rates

Pattern among Lineages \_\_\_\_\_ Same (Homogeneous)

Data Subset to Use

Gaps/Missing Data Treatment \_\_\_\_\_ Pairwise deletion

Codons Included \_\_\_\_\_ 1st+2nd+3rd+Non-Coding

No. of Sites : 633

No Of Bootstrap Reps = 1000

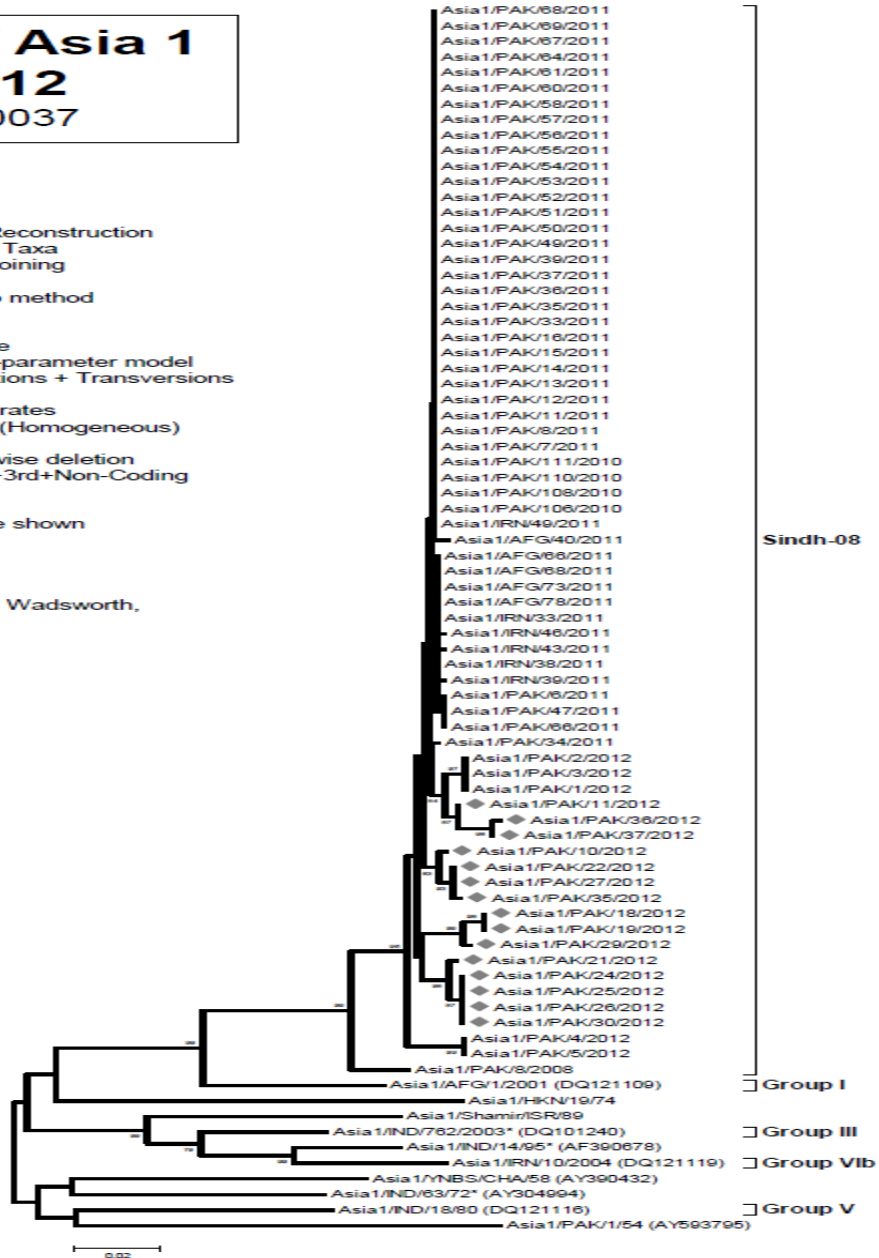
Only bootstrap values of 70% and above are shown

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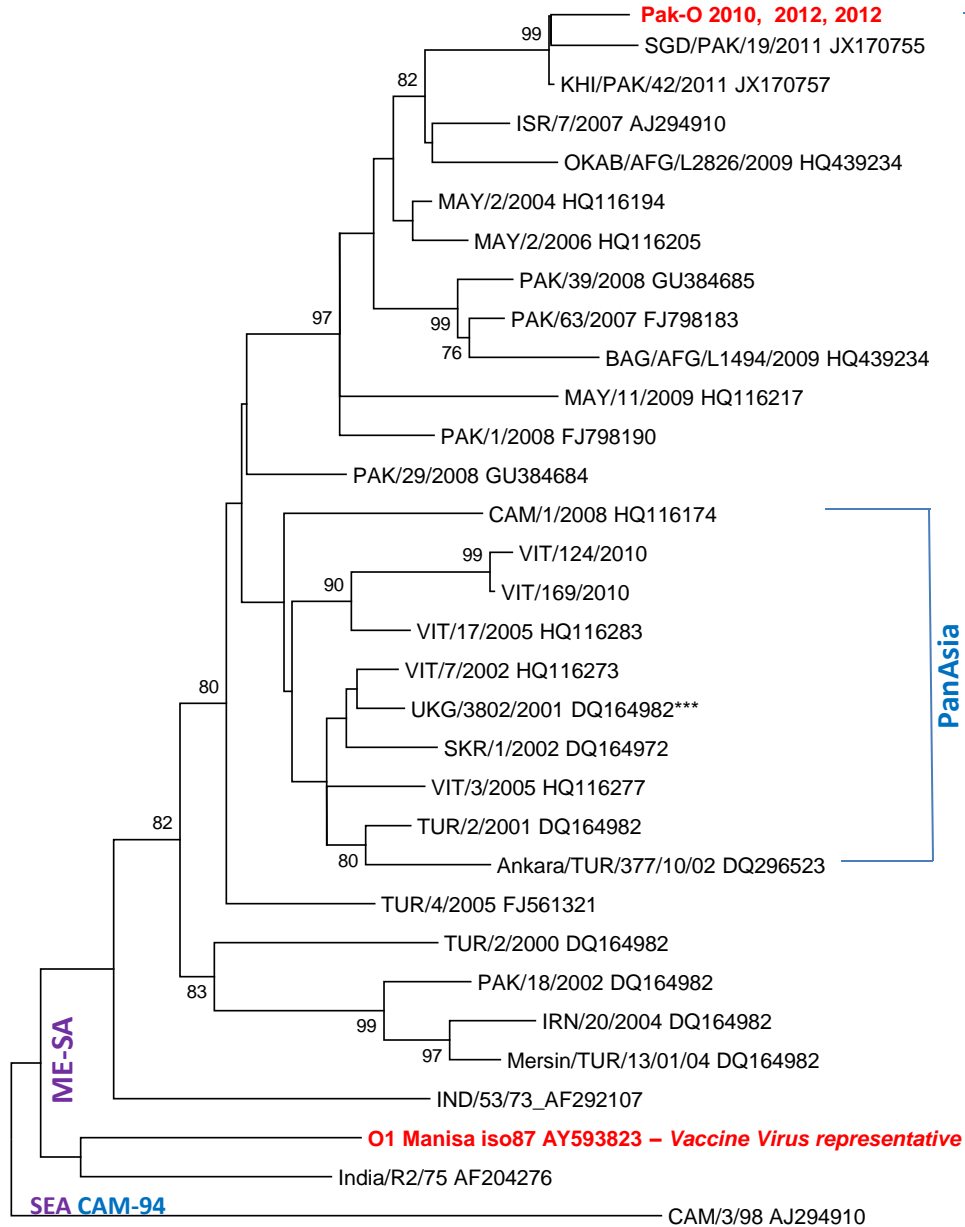
# Research Collaboration

Plum Island Animal Disease Center, New York

## Objectives

- To better understand the epidemiology of FMDV strains circulating in Pakistan
- To determine the phylogenetic relationships of FMDV strains in Pakistan (Serotype A, O and Asia1)
- To determine the antigenic relationship (vaccine matching) of FMDV strains

# Serotype O FMDV

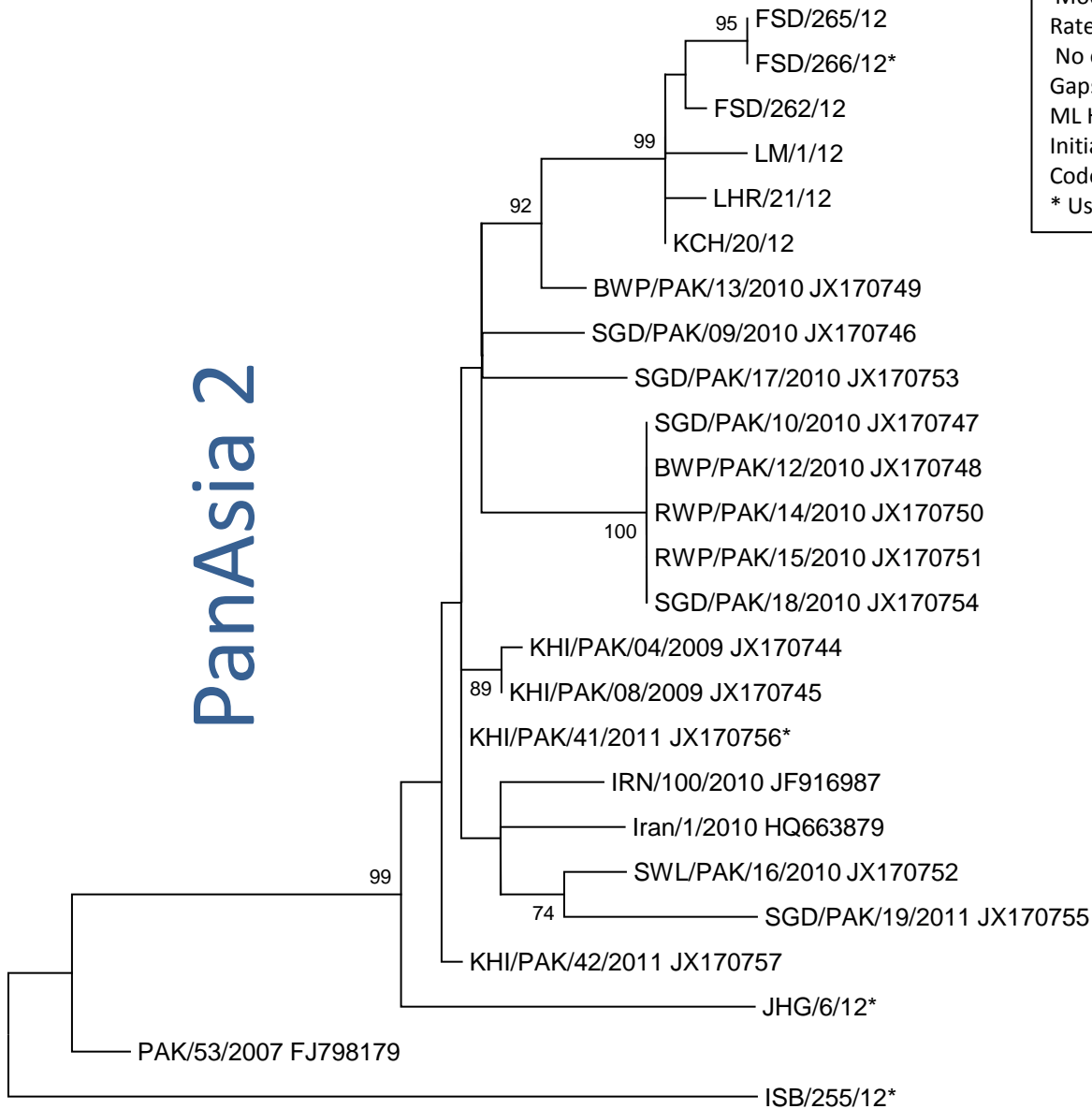


## Phylogeny Reconstruction

- Statistical Method ----- Maximum Likelihood
- Test of Phylogeny ----- Bootstrap method (500 replicates)
- Substitutions Type ----- Nucleotide
- Model/Method ----- General Time Reversible model
- Rates among Sites ----- Gamma distributed with Invariant sites (G+I)
- No of Discrete Gamma Categories - 5
- ML Heuristic Method ----- Nearest-Neighbor-Interchange (NNI)
- Initial Tree for ML ----- Make initial tree automatically
- No. of Seqs : 33 No. of Sites : 623
- \*\*\* - reference strain

# Serotype O FMDV

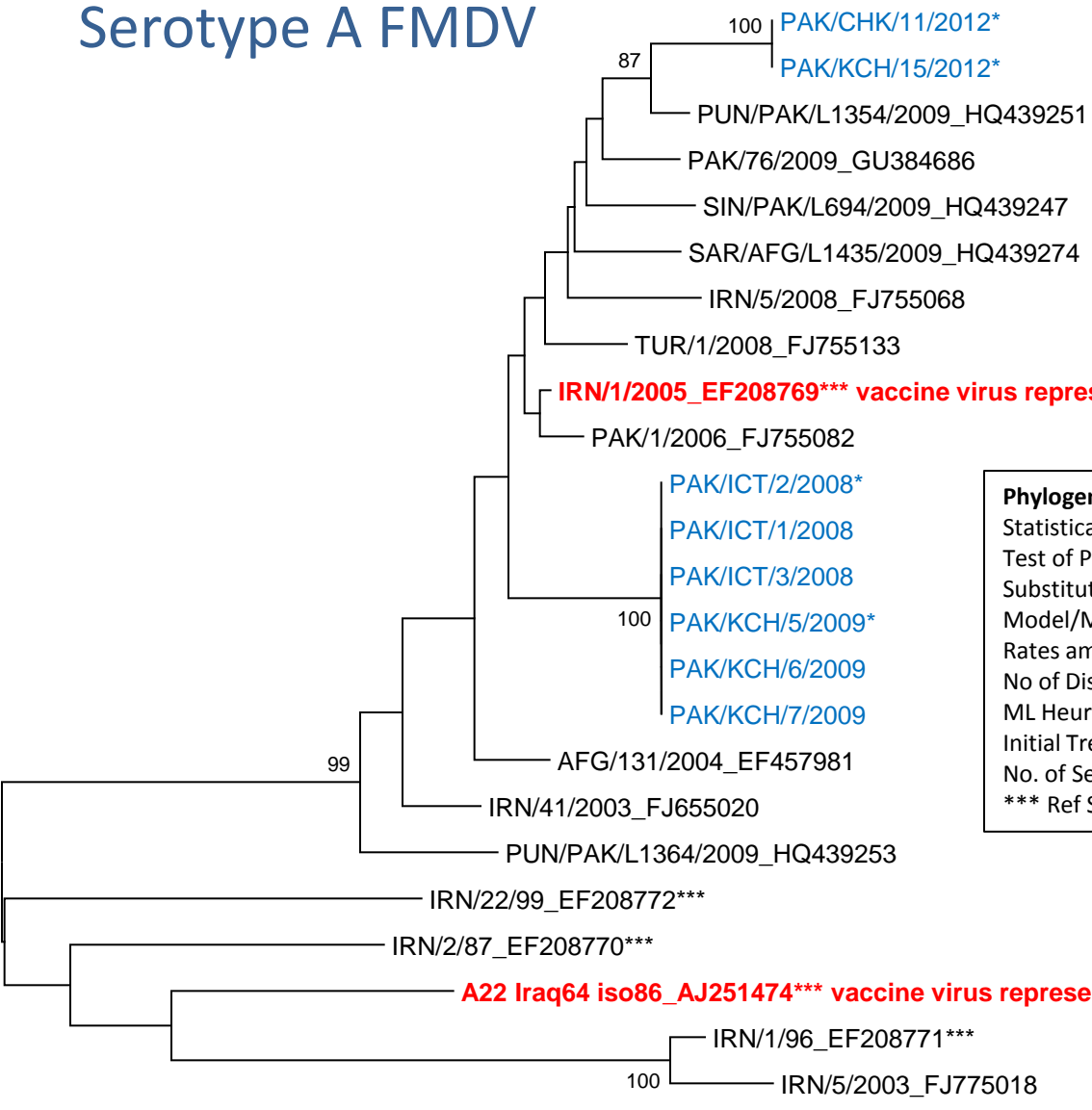
## PanAsia 2



Phylogeny Reconstruction  
 Statistical Method ----- Maximum Likelihood  
 Test of Phylogeny ----- Bootstrap method (500 rep)  
 Substitutions Type ----- Nucleotide  
 Model/Method ----- General Time Reversible model  
 Rates among Sites ----- Gamma distributed with Invariant sites  
 No of Discrete Gamma Categories - 5  
 Gaps/Missing Data Treatment ----- Complete deletion  
 ML Heuristic Method ----- Nearest-Neighbor-Interchange (NNI)  
 Initial Tree for ML ----- Make initial tree automatically  
 Codons Included ----- 1st+2nd+3rd+Non-Coding  
 \* Used for vaccine matching

0.01

# Serotype A FMDV

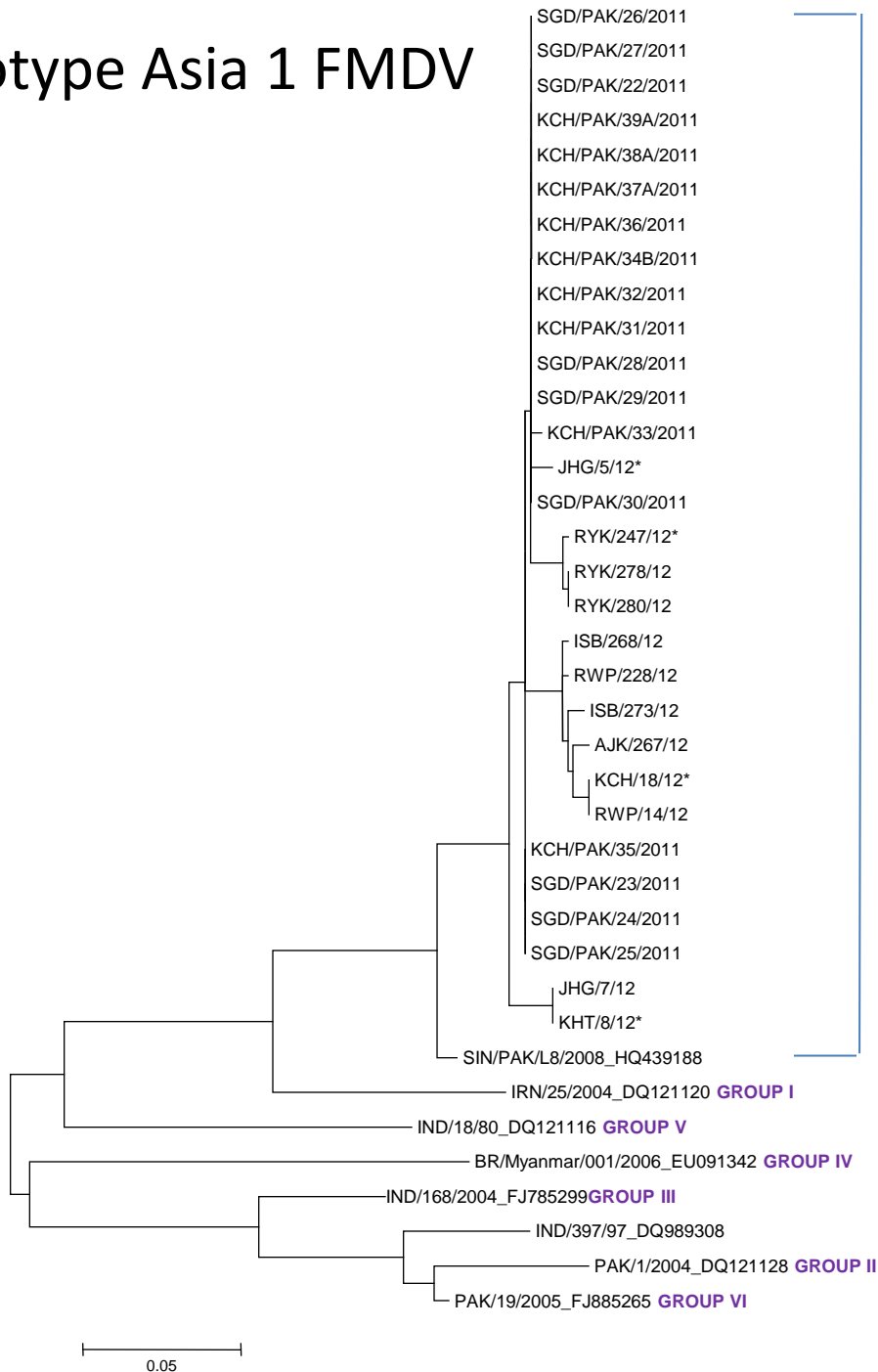


**Phylogeny Reconstruction**

Statistical Method ----- Maximum Likelihood  
 Test of Phylogeny ----- Bootstrap method (500 rep)  
 Substitutions Type ----- Nucleotide  
 Model/Method ----- General Time Reversible model  
 Rates among Sites ----- Gamma distributed with Invariant sites (G+I)  
 No of Discrete Gamma Categories - 5  
 ML Heuristic Method ----- Nearest-Neighbor-Interchange (NNI)  
 Initial Tree for ML ----- Make initial tree automatically  
 No. of Seqs : 24 No. of Sites : 623  
 \*\*\* Ref Strains \* used for vaccine matching

0.05

# Serotype Asia 1 FMDV



## GROUP VII

### Phylogeny Reconstruction

Statistical Method ----- Maximum Likelihood  
 Substitutions Type ----- Nucleotide  
 Model/Method ----- General Time Reversible model  
 Rates among Sites ----- Gamma distributed with Invariant sites (G+I)  
 No of Discrete Gamma Categories - 5  
 Gaps/Missing Data Treatment ----- Complete deletion  
 ML Heuristic Method ----- Nearest-Neighbor-Interchange (NNI)  
 Initial Tree for ML ----- Make initial tree automatically  
 No. of Seqs : 38 No. of Sites : 626  
 \* Used for vaccine matching

# Vaccine Matching Approach

1. **ARRIAH Vaccine used in the FAO project was inoculated to raise sera in seronegative calves. Booster dose given after 30 days and serum collected 30 days post booster. This sera was used for vaccine matching**
2. **Vaccine was trivalent and contained A Iran 2005, O PanAsia 2 and Asia-1 Shamir (not less than 6 PD<sub>50</sub>)**
3. **Homologous or nearest neighbor strains to vaccine viruses used for vaccine matching: *O1 Manisa, Asia1 Shamir, A IRN-05, A22 IRQ* (PanAsia2 vaccine virus could not be used as it was not available at PIADC)**

| <b>WRL</b>               | <b>ARS</b>                |
|--------------------------|---------------------------|
| Monovalent Vaccine       | Trivalent Vaccine         |
| IB-RS-2 cells            | LFBK cells                |
| Single dose vaccine sera | Booster dose vaccine sera |
| Cattle Sera only         | Cattle and Buffalo Sera   |
| Pooling of five sera     | Pooling of five sera      |

# Serotype O Vaccine Matching against O1 Manisa

| O/ISB/255/12 |       |          | O/FSD/266/12 |       |          | O/JGH/6/12 |       |          | O/KHI/41/11 |       |          |
|--------------|-------|----------|--------------|-------|----------|------------|-------|----------|-------------|-------|----------|
| BT           | neut. | r1-value | BT           | neut. | r1-value | BT         | neut. | r1-value | BT          | neut. | r1-value |
| 2.33         | 1.8   | 0.71     | 2.18         | 1.65  | 0.5      | 2.48       | 1.95  | 1        | 2.25        | 1.95  | 1        |
| 1.8          | 1.5   | 0.5      | 2.4          | 1.95  | 0.5      | 1.5        | 1.5   | 0.5      | 2.03        | 1.35  | 0.35     |
|              |       |          | 1.5          | 1.8   | 1        |            |       |          |             |       |          |

- BT = Back Virus Titration
- All values given in  $\log_{10}$

• **r1-values** are suggesting that the vaccine is protective against the serotype O viruses tested.

# Serotype A Vaccine Matching against A-IRN05 and A22 IRQ

\* All values given in  $\log_{10}$

\*\* homologous strains failed on virus back titration

BT = Back Virus Titration

| A/ICT/2/2008 |       |        |        | A/KCH/5/2009 |       |        |        | A/CHK/11/2012 |      | A/KCH/15/12 |      |
|--------------|-------|--------|--------|--------------|-------|--------|--------|---------------|------|-------------|------|
| BT           | neut. | r1 A22 | R1 IRN | BT           | neut. | r1 A22 | r1 IRN | BT            | neut | BT          | neut |
| 1.65         | 1.65  | 1.41** | 1**    | 1.73         | 1.95  | 1.41   | 1      | 1.8           | <1.2 | 1.95        | <1.2 |
| 2.03         | 1.65  | 0.7    | 0.25   | 2.25         | 1.65  | 1.41   | 0.5    | 2.03          | <1.2 | 2.48        | <1.2 |
|              |       |        |        |              |       |        |        |               |      | 1.58        | <1.2 |

- VN titers above 1.6 observed for 2008-2009 isolates
- VN titers below 1.2 for 2012 isolates, suggesting that the vaccine is not a good match to these strains. However, sample size is too small to make an accurate conclusion.

# Serotype Asia1 Vaccine Matching against Asia1 Shamir

| As1/RYK/247/12 |       |          | As1/KCH/18/12 |       |          | As1/KHT/8/12 |       |          | As1/JHG/5/12 |       |          |
|----------------|-------|----------|---------------|-------|----------|--------------|-------|----------|--------------|-------|----------|
| BT             | neut. | r1-value | BT            | neut. | r1-value | BT           | neut. | r1-value | BT           | neut. | r1-value |
| 1.95           | 1.2   | n.d.     | 2.33          | 1.2   | n.d.     | 2.25         | 1.5   | n.d.     | 2.25         | 1.5   | n.d.     |
| 2.48           | <1.2  | n.d.     | 2.55          | <1.2  | n.d.     | 1.5          | <1.2  | n.d.     | 1.5          | <1.2  | n.d.     |
| 2.03           | 1.35  | 0.25     | 1.5           | 1.35  | 0.25     | 2.55         | 1.2   | 0.18     | 2.25         | 1.2   | 0.18     |
|                |       |          | 2.25          | 1.8   | 0.7      |              |       |          |              |       |          |

- BT = Back Virus Titration
- All values given in  $\log_{100}$

- VN titers generally low (<1.2) Asia1 strains suggesting not a good vaccine match

# FMD Vaccines Available in Pakistan

- **Locally Manufactured Vaccines**

- FMD Vaccine, VRI, Lahore: **trivalent (strains not known), formalin inactivation, alum-ppt.**
- F.M. CENNA, Avicenna Labs, Sheikhpura: **trivalent (strains not known), formalin inactivation, alum-ppt**
- FMD Vaccine, UVAS, Lahore: **trivalent, BEI inactivation, double oil emulsion**

- **Imported Vaccines**

- Aftovaxpur, Merial, UK: **trivalent (strains not mentioned)**
- Aftebin, Czech Republic: **bivalent (strains not mentioned)**
- FMD Vaccine, ARRIHA, Russia: **trivalent (A Iran/05, O PanAsia II, Asia-1 Shamir)**
- Decivac FMD DOE, Intervet, Germany: **trivalent (A Tur/06, O Tur/09, Asia-1 Shamir) double oil emulsion**

- **Informally available**

- Raksha Ovac, India: **trivalent (strains not mentioned), double oil emulsion**
- FMD Vaccine, Razi Institute, Iran: **trivalent (strains not mentioned)**

# Vaccination in dairy colony production system

- Control of FMD through vaccination being demonstrated under FAO-GOP-USDA project. Vaccine used is specially formulated vaccine by ARRIAH, Russia (not less than 6 PD<sub>50</sub> for each strain) and contains O sub-serotype PanAsia-II, A sub-serotypes Iran05 and Asia-1 sub-serotype Shamir
- Dairy colonies where vaccination is being carried out are:
  - Khalsa area Peshawar dairy colony
  - Landhi Dairy colony and Nagori Dairy Colony, Karachi
  - Rakh Chandra Gwala Colony, Lahore
  - Eastern bypass and Quarry Road, Quetta
  - Livestock farms, Mirpur
  - Suhan and Tarlai Farms, Islamabad
- Tagging carried out in all vaccinated animals
- Primary, booster dose after one month and then six monthly vaccination
- New entrants in the dairy colonies also being tagged and vaccinated
- Regular monitoring (clinical and serological) for FMD infection

# Vaccination in dairy colonies

| <b>Dairy Colony</b>                                      | <b>Farms #</b> | <b>Animals #</b> |
|--|----------------|------------------|
| <b>Khalsa area Peshawar</b>                              | <b>330</b>     | <b>10000</b>     |
| <b>Landhi Cattle colony, Karachi</b>                     | <b>27</b>      | <b>6888</b>      |
| <b>Nagori Farmers Dairy Cooperative Society, Karachi</b> | <b>28</b>      | <b>4961</b>      |
| <b>Rakh Chandra Gwala Colony, Lahore</b>                 | <b>183</b>     | <b>7014</b>      |
| <b>Eastern bypass, Quetta</b>                            | <b>16</b>      | <b>3927</b>      |
| <b>Quary Road, Quetta</b>                                | <b>33</b>      | <b>7567</b>      |
| <b>Livestock farms, Mirpur</b>                           | <b>30</b>      | <b>945</b>       |
| <b>Suhan and Tarlai, Islamabad</b>                       | <b>278</b>     | <b>6529</b>      |
| <b>Total</b>   | <b>925</b>     | <b>47831</b>     |

## ***Immunization in rural smallholder dairy production system***

- **Selection of villages for vaccine trial undertaken in consultation with respective livestock departments**
- **Each livestock department identified 3 areas in their province / region**
- **Baseline survey for all villages was undertaken**
- **Vaccination (primary , booster and six monthly) done**
- **Tagging done in young animals (< 1 year age)**
- **Calves reaching 4 months age also being vaccinated**
- **Regular monitoring (clinical and serological) for FMD infection being undertaken**
- **No evidence of clinical disease in vaccinated animals. Sera to be analyzed**

# Vaccination in Smallholders Rural Production System

| District       | Villages # | Farmers # | Animals # |
|----------------|------------|-----------|-----------|
| Rahim Yar Khan | 5          | 810       | 7014      |
| Jhang          | 4          | 704       | 4189      |
| Attock         | 3          | 910       | 5000      |
| Nausheroferoz  | 24         | 722       | 3314      |
| Tando Allahyar | 12         | 689       | 3314      |
| Thatta         | 8          | 210       | 3650      |
| Matiari        | 5          | 543       | 5590      |
| Abbottabad     | 5          | 1199      | 4702      |
| DI Khan        | 5          | 618       | 4990      |
| Nowshera       | 5          | 590       | 4195      |

# Vaccination in Smallholders Rural Production System

| District       | Villages # | Farmers #    | Animals #    |
|----------------|------------|--------------|--------------|
| Pishin         | 4          | 295          | 3207         |
| Lasbella       | 4          | 346          | 2818         |
| Bolan          | 6          | 290          | 3230         |
| Muzaffarabad   | 6          | 2288         | 3480         |
| Mirpur         | 14         | 1002         | 3451         |
| Rawalakot      | 11         | 2768         | 3874         |
| Ghizer         | 4          | 910          | 7000         |
| Gilgit         | 4          | 750          | 5177         |
| Skardu         | 4          | 1012         | 2928         |
| Mohmand Agency | 5          | 664          | 3816         |
| Bajur Agency   | -          | -            | -            |
| FR DI Khan     | -          | -            | -            |
| <b>Total</b>   | <b>138</b> | <b>17320</b> | <b>84939</b> |

# FMD Vaccination in Yak

- Yak are special to high mountain areas and are important in livelihood of the communities
- Trial started in Ghizer district (9 villages)
- Total Yak population of these villages is 3417
- Vaccinated group is 2193 while unvaccinated group is 1224 yaks.
- 2396 yaks tagged and 2615 vaccinated (community enthusiastic)
- Blood samples taken from yak for sero-surveillance (66 out of 136 were positive for FMD-NSP antibodies before vaccination)
- Vaccine response (775 animals bled) and outbreak data being recorded
- No clinical outbreak recorded in vaccinated animals



# Acknowledgements



FAS for Financial Assistance, ARS (Dr Luis Rodriguez, Dr. Zaheer Ahmed and Dr. Anna Ludi) for research Collaboration



Animal Health Programme, PARC for research (Dr Khalid Naeem, Dr. Umer Farooq, Dr Naila Siddique)



Animal Husbandry Commissioner Office, National Veterinary Labs, Provincial / Regional Livestock Departments, Public and Private vets and Livestock farmers



Team of GCP/PAK/123/USA Project Progressive Control of FMD in Pakistan (Dr. M. Afzal, Dr. Manzoor Hussain, Dr. Ehtisham Khan, Dr. Javed Arshad, Dr. Nasrullah Panhwer and Dr. Aftab Ahmad)

# Homologous or nearest neighbor strains to vaccine viruses for vaccine matching

## Lineages\*

- *O1 Manisa\*\**
- *Asia1 Shamir*
- *A IRN-05*
- *A22 IRQ*

\* Specific strains used in vaccine formulation is not known and unavailable

\*\* Currently do not have the PanAsia2 vaccine virus, O1 Manisa is closest relative available to us.

# Reference sera generated using ARRIAH trivalent Vaccine VN titers

| Animal #      |         | O1 Manisa   | Asia1 Shamir | A22 Iraq    | A Iran-05   |
|---------------|---------|-------------|--------------|-------------|-------------|
| 20.00         | Cattle  | 2.62        | 1.41         | 1.79        | 1.95        |
| 22.00         |         | 2.23        | <1.2         | 1.86        | 1.65        |
| 23.00         |         | 2.23        | 1.57         | 1.41        | <1.2        |
| 177.00        |         | 2.55        | 2.40         | 1.71        | 1.65        |
| 401.00        |         | 1.71        | <1.2         | <1.2        | <1.2        |
| <b>587.00</b> | Buffalo | <1.2        | <1.2         | <1.2        | <1.2        |
| <b>588.00</b> |         | <b>1.34</b> | <1.2         | <1.5        | <b>1.50</b> |
| <b>590.00</b> |         | <1.2        | <b>1.62</b>  | <1.35       | <b>1.20</b> |
| <b>592.00</b> |         | <b>1.71</b> | <1.2         | <1.2        | <1.2        |
| <b>593.00</b> |         | <b>1.64</b> | <1.2         | <1.35       | <1.2        |
| <b>594.00</b> |         | <1.2        | <1.2         | <b>1.20</b> | <1.2        |

- All values given in  $\log_{10}$

# ARRIAH Sera

## Pooled

- ❖ Reference sera was made using the five cattle sera  
→ This sera was used for all subsequent tests

| Pooled Sera      | O1 Manisa |       | Asia1 Shamir |       | A22 Iraq |       | A Iran-05 |       |
|------------------|-----------|-------|--------------|-------|----------|-------|-----------|-------|
|                  | BT        | Neut. | BT           | Neut. | BT       | Neut. | BT        | Neut. |
| animal ID 20-401 |           |       |              |       |          |       |           |       |
| Repetition 1     | 1.88      | 1.95  | 1.65         | 1.95  | 2.25     | 1.8   | 1.73      | 1.95  |
| Repetition 2     | 2.4       | 2.25  | 2.25         | 1.95  | 2.33     | 1.5   | 1.33      | 1.95  |
| Repetition 3     | 2.03      | 1.8   |              |       | 1.73     | 1.5   | 1.65      | 1.65  |

- All values given in  $\log_{10}$
- BT = Back Virus Titration