DETECTION OF FMDV IN BUFFALO *(BUBALUS BUBALIS)* POPULATION IN THREE PROVINCES OF PAKISTAN

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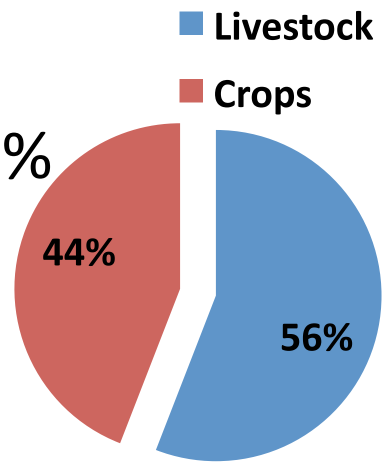
Role of Livestock in the Economics of Pakistan



- Share of Agriculture in GDP: 21.0%
- Share of Livestock in agriculture : 56.3 %
- Share of Livestock in GDP: 11.8%
- Main source of labour: 43.7%
- Earnings through Livestock sector: Rs. 776.5 billion (2014-15)
- Livestock progress: 2.9% annually (2014-15)

LIVESTOCK POPULATION IN PAKISTAN-2015

Species	Population (Million Nos.)	Milk Production (000 tones)
Cattle/Cow	39.7	18,027
Buffalo	34.6	31,252
Sheep	29.1	38
Goat	66.6	822
Total	170.0	50,990

Source: Ministry of National Food Security & Research, Govt. of Pakistan

FMD IN PAKISTAN: IMPACT ON ECONOMY

FMDV strains in Pakistan: O, A and Asia 1

Losses due to FMD in Pakistan Rs ~20.5 Billion

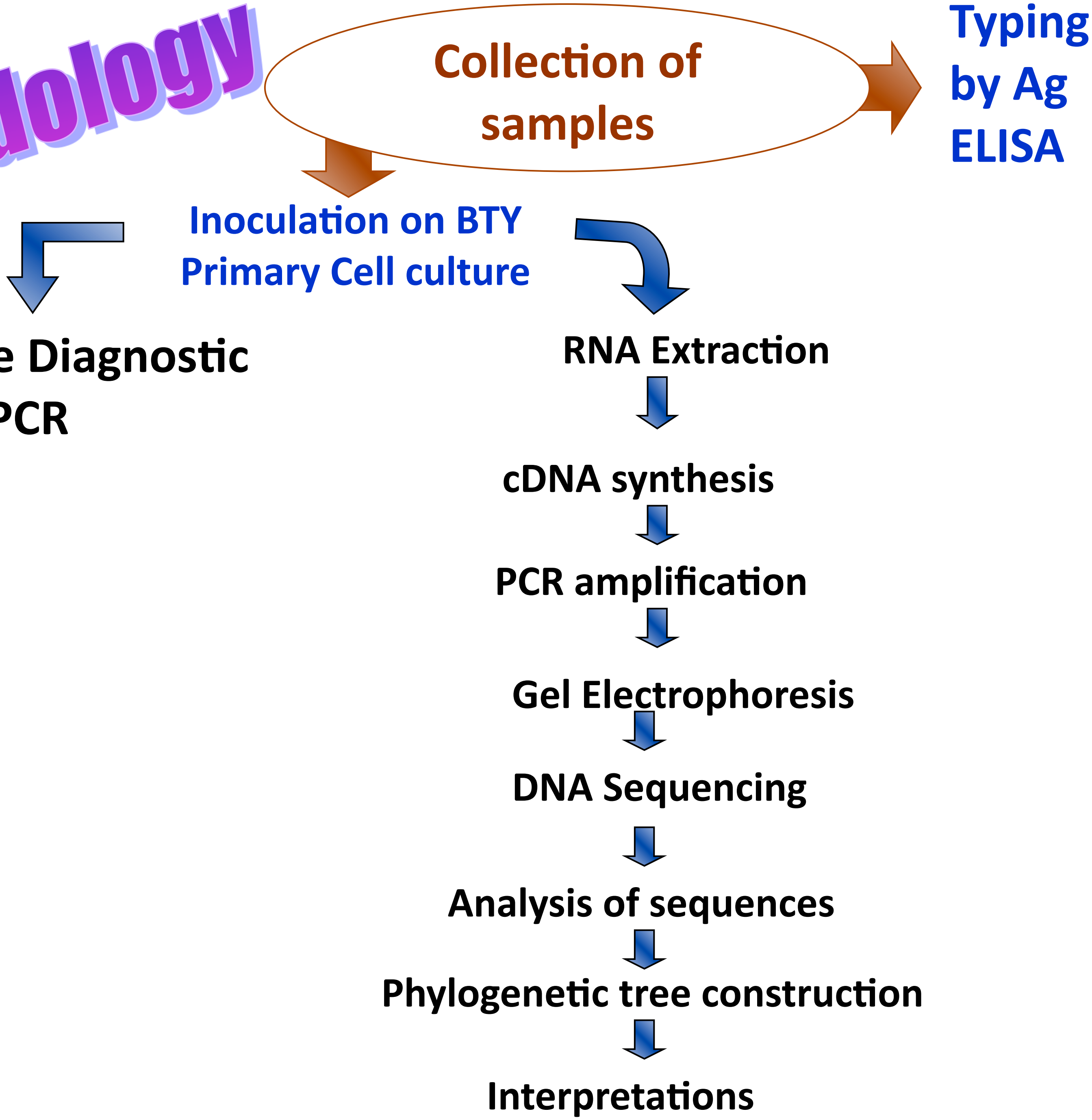
The economic losses are due to:

- **Direct Losses** (Deaths, Loss of production draught capacity)
- **Indirect Losses** (Restrictions on trade, import and export of live animals and dairy products)
- **Treatment and vaccination costs**

Areas of sample collections

1. Punjab province
2. Khyber Pakhtunkhawa
3. Balouchistan

Methodology



Collection of samples

Typing by Ag ELISA

Inoculation on BTY Primary Cell culture

Real Time Diagnostic PCR

RNA Extraction

cDNA synthesis

PCR amplification

Gel Electrophoresis

DNA Sequencing

Analysis of sequences

Phylogenetic tree construction

Interpretations

Virus Isolation and Antigen-ELISA

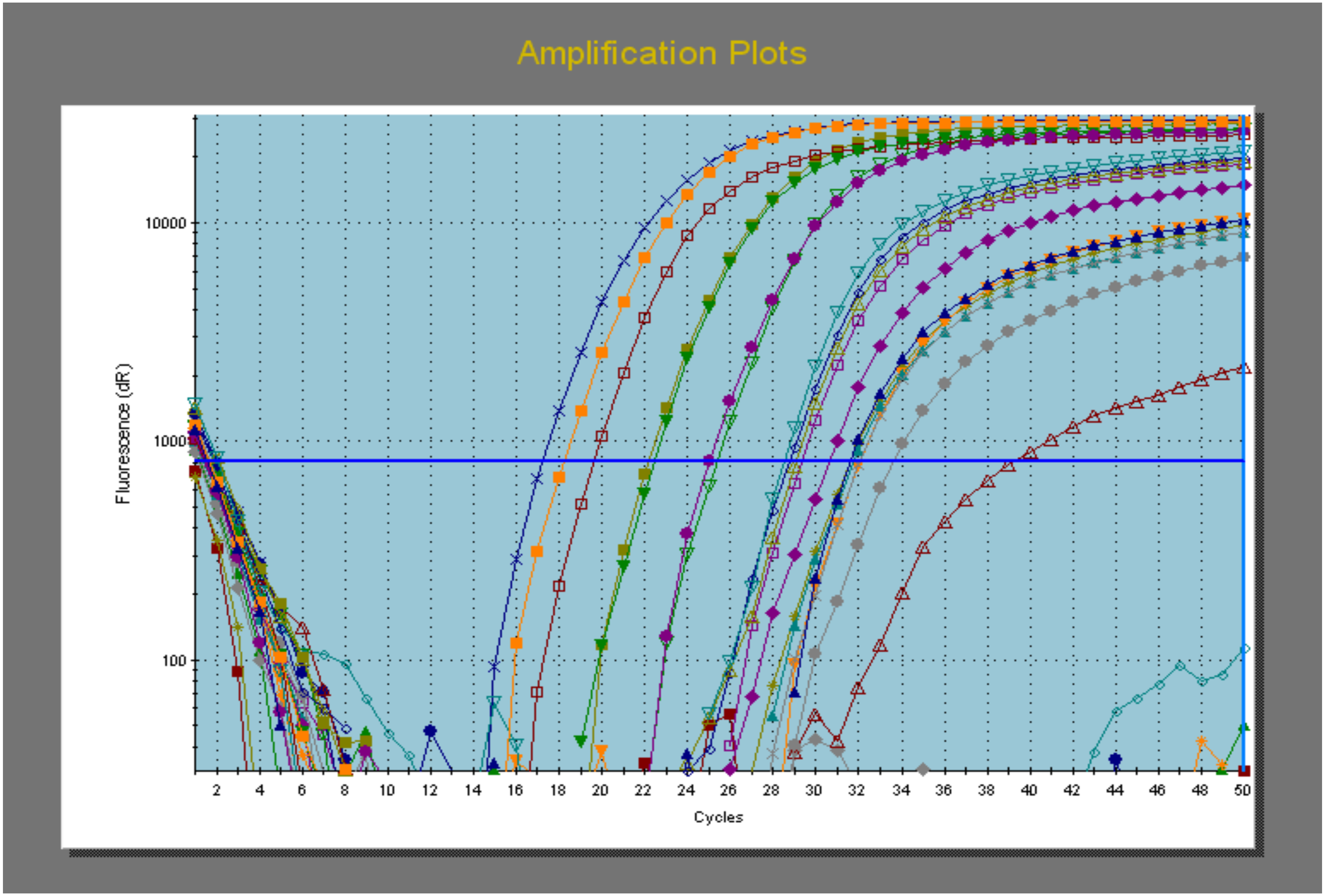
Virus isolation - BTY primary cell culture
IB-RS-2

Ag-ELISA - For identification of foot-and-mouth disease virus strain in the sample

REAL TIME RT-PCR (rRT-PCR)

		Oligo name	Sequence (5’ – 3’)
FMDV 5’UTR	Forward Primer	SA-IR-219-246F	CAC YTY AAG RTG ACA YTG RTA CTG GTA C
	Reverse Primer	SA-IR-315-293R	CAG ATY CCR AGT GWC ICI TGT TA
	Taqman PROBE	SAmulti2-P-IR-292-269R	CCT CGG GGT ACC TGA AGG GCA TCC
FMDV 3D	Forward Primer	Callahan 3DF	ACT GGG TTT TAC AAA CCT GTG A
	Reverse Primer	Callahan 3DR	GCG AGT CCT GCC ACG GA
	Taqman PROBE	Callahan 3DP	TCC TTT GCA CGC CGT GGG AC

REAL TIME RT-PCR (rRT-PCR): RESULTS



REVERSE TRANSCRIPTASE PCR (RT-PCR)

i. RNA ISOLATION

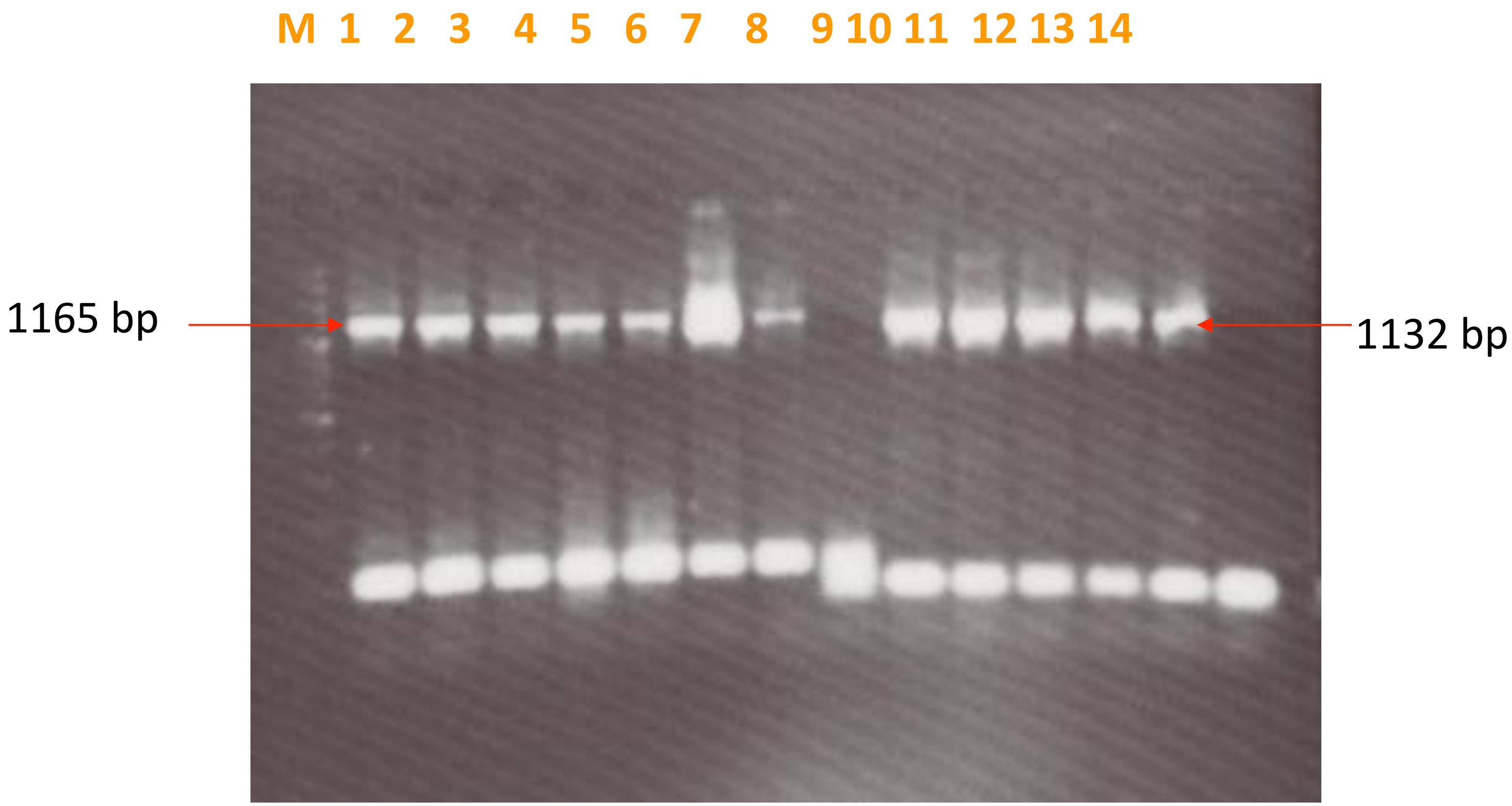
ii. cDNA SYNTHESIS

iii. PCR AMPLIFICATION

RT-PCR STRAIN SPECIFIC PRIMERS For Type O

Primer	Oligo Name	Sequence 5’ to 3’	Product Size (bp)
Forward Primer	**O-C244F**	GCAGCAAAACACATGTCAAACACCTT	1162-1165
Forward Primer	**O1C272F**	TBGCRGGNCTYGCCCAGTACTAC	1132-1135
Reverse Primer	**EUR 2B52R**	GACATGTCCTCCTGCATCTGGTTGAT	

RT-PCR RESULTS

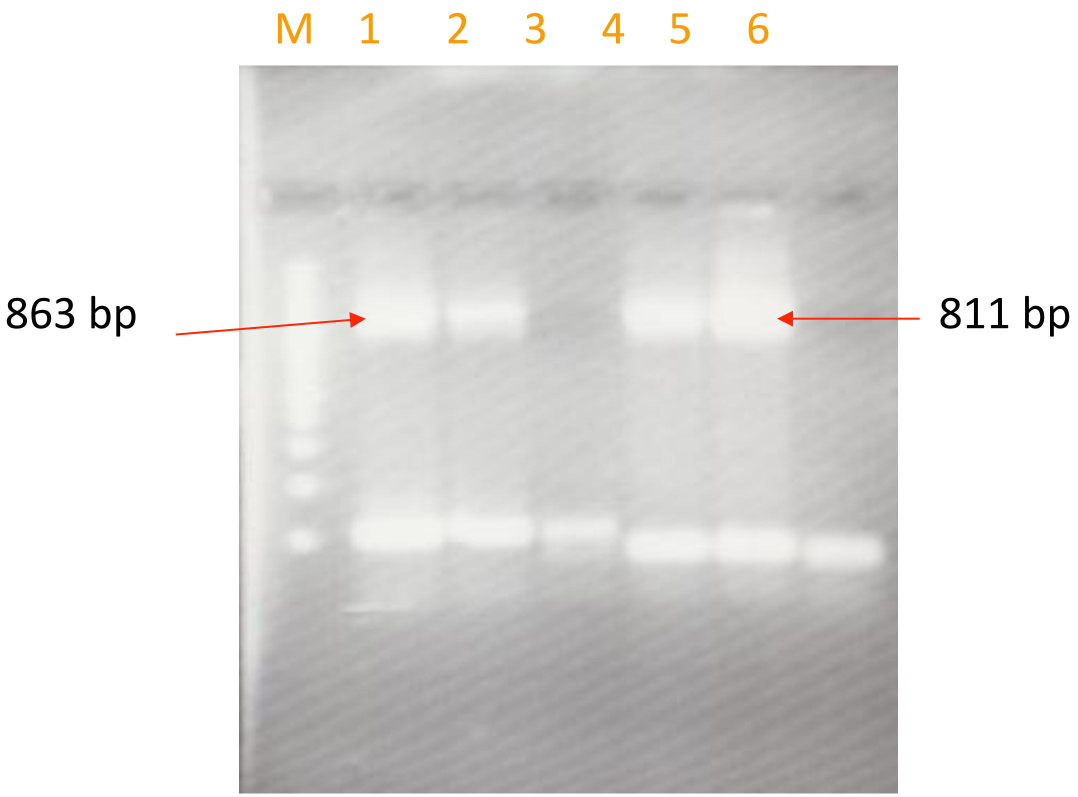


M Marker (100 bp), **1**. O-Pak 31/05A, **2**. O-Pak 32/05A, **3**. O-Pak 33/06A,
4. O-Pak 34/05A, **5**. O-Pak 35/05A, **6**. O-Pak 38/06A, **7**. O-Pak 60/06A,
8. Neg control **9**. O-Pak 31/05B, **10**. O-Pak 31/05B, **11**. O-Pak 32/05B,
12. O-Pak 33/05B, **13**. O-Pak 34/05B, **14**. Neg control

RT-PCR STRAIN SPECIFIC PRIMER FOR FMDV A

Primer	Oligo Name	Sequence 5’ to 3’	Product Size (bp)
Forward Primer	A-1C562F	TACCAAATTACACACGGGAA	863-866
Forward Primer	A-1C612F	TAGCGCCGGCAAAGACTTTGA	811-814
Reverse Primer	EUR2B52R	GACATGTCCTCCTGCATCTGGTTGAT	

RT-PCR RESULTS



M Marker (100 bp), **1**. FMDV A VS 522A, **2**. FMDV A VS 523A, **3**. Neg. Control, **4**. FMDV A VS 522B, **5**. FMDV A VS 523B, **6**. Neg. Control

SEQUENCING OF PCR PRODUCTS

Accession No.	FMDV Strain	Location
FJ798162	O	VP1
FJ798163	O	VP1
FJ798164	O	VP1
FJ798165	O	VP1
FJ798166	O	VP1
FJ798167	O	VP1
FJ798168	O	VP1
FJ798169	O	VP1
FJ798170	O	VP1
FJ798171	O	VP1
FJ798172	O	VP1
FJ798173	O	VP1
FJ798174	O	VP1
FJ798175	O	VP1
FJ798176	O	VP1
FJ798177	O	VP1
FJ798178	O	VP1
FJ798179	O	VP1
FJ798180	O	VP1
FJ798181	O	VP1

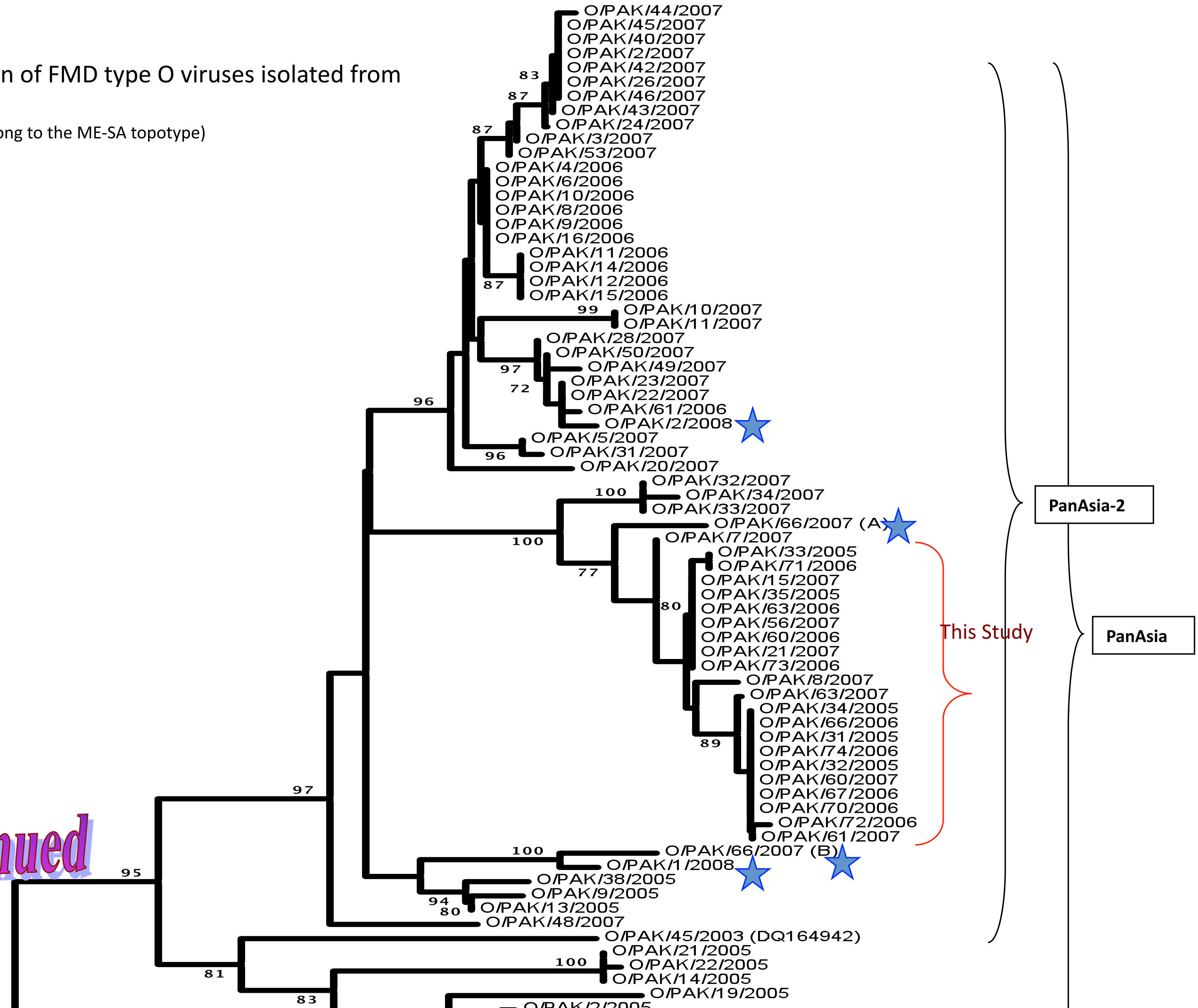
SEQUENCING OF PCR PRODUCTS (CONTINUED)

FJ798182	O	VP1
FJ798183	O	VP1
FJ798184	O	VP1
FJ798185	O	VP1
FJ798186	O	VP1
FJ798187	O	VP1
FJ798188	O	VP1
FJ798189	O	VP1
FJ798190	O	VP1
FJ798191	O	VP1
FJ798192	O	VP1
FJ798193	O	VP1
FJ798194	A	VP1
Accession No.	**FMDV Strain**	**Location**
GU384682	O	Whole Genome
GU384683	O	Whole genome
GU384684	O	P1
GU384685	O	P1
GU384686	A	P1

PHYLOGENETIC ANALYSIS OF FMDV ‘O’

Comparison of FMD type O viruses isolated from Pakistan

(all viruses belong to the ME-SA topotype)



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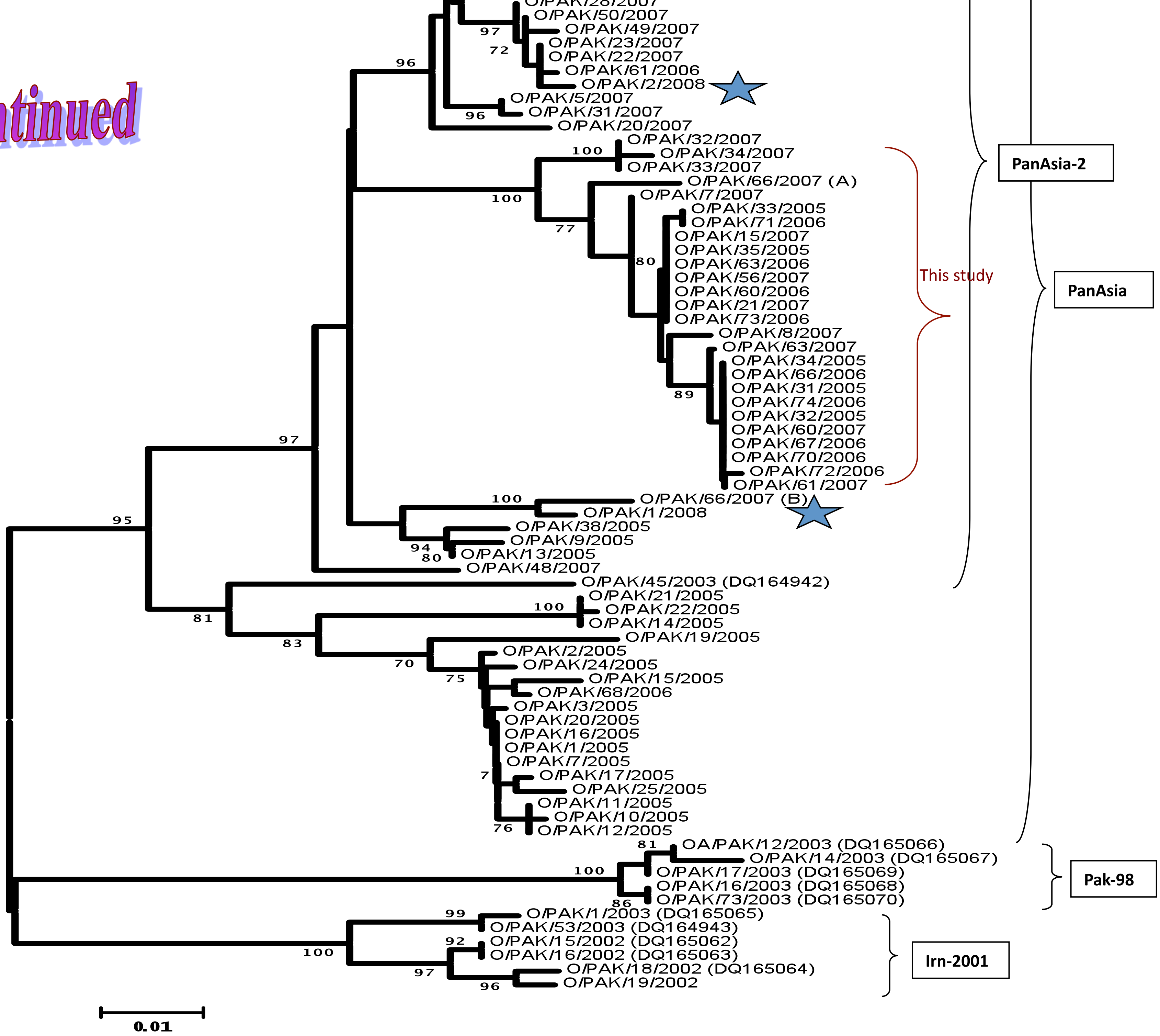
O/PAK/28/2007
O/PAK/50/2007
O/PAK/49/2007
O/PAK/23/2007
O/PAK/22/2007
O/PAK/61/2006
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O/PAK/20/2007
O/PAK/32/2007
O/PAK/34/2007
O/PAK/33/2007
O/PAK/66/2007 (A)
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O/PAK/16/2002 (DQ165063)
O/PAK/18/2002 (DQ165064)
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PanAsia
Pak-98
Irn-2001
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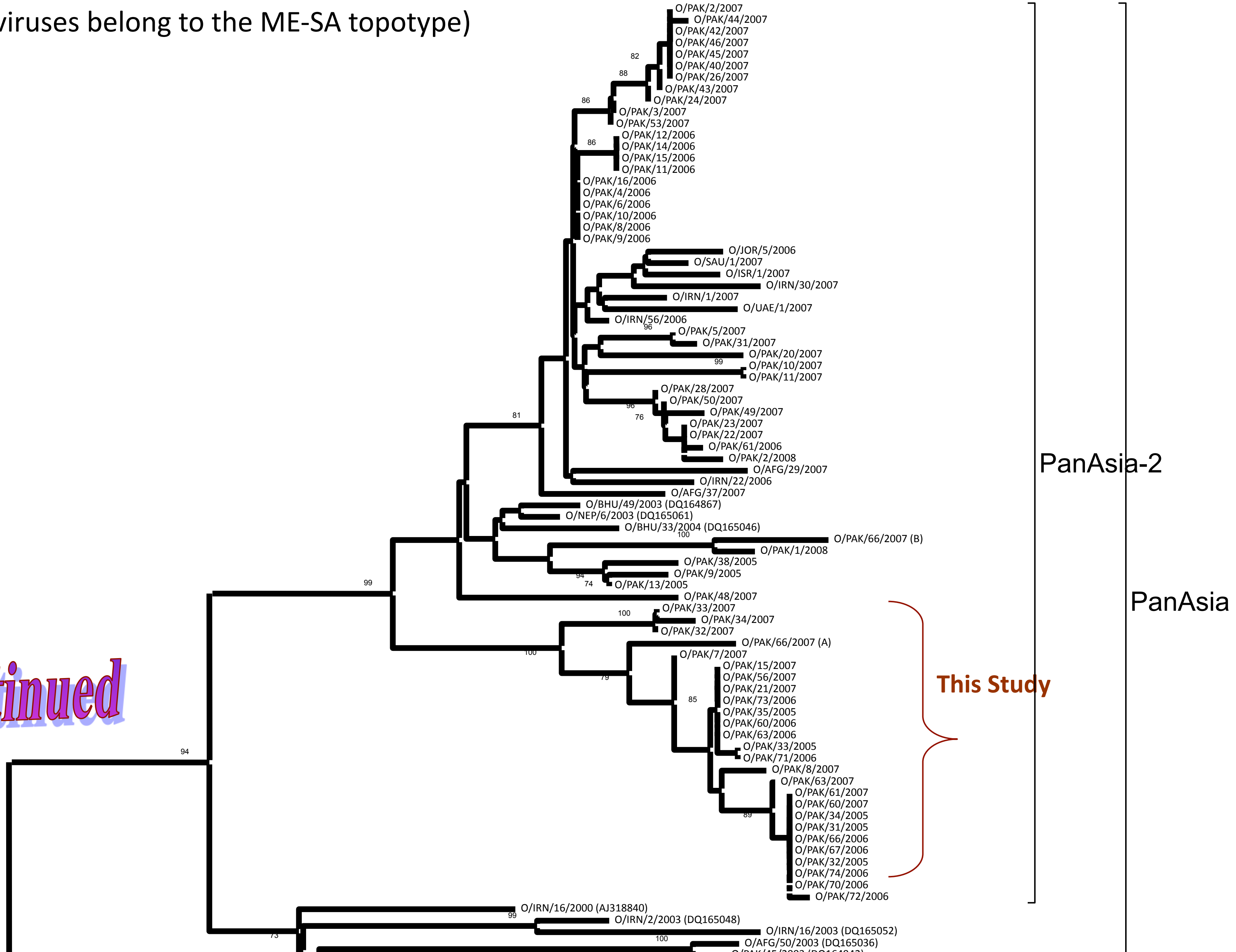
PHYLOGENETIC ANALYSIS

Comparison of FMD type O viruses isolated from Pakistan
(all viruses belong to the ME-SA topotype)



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Results

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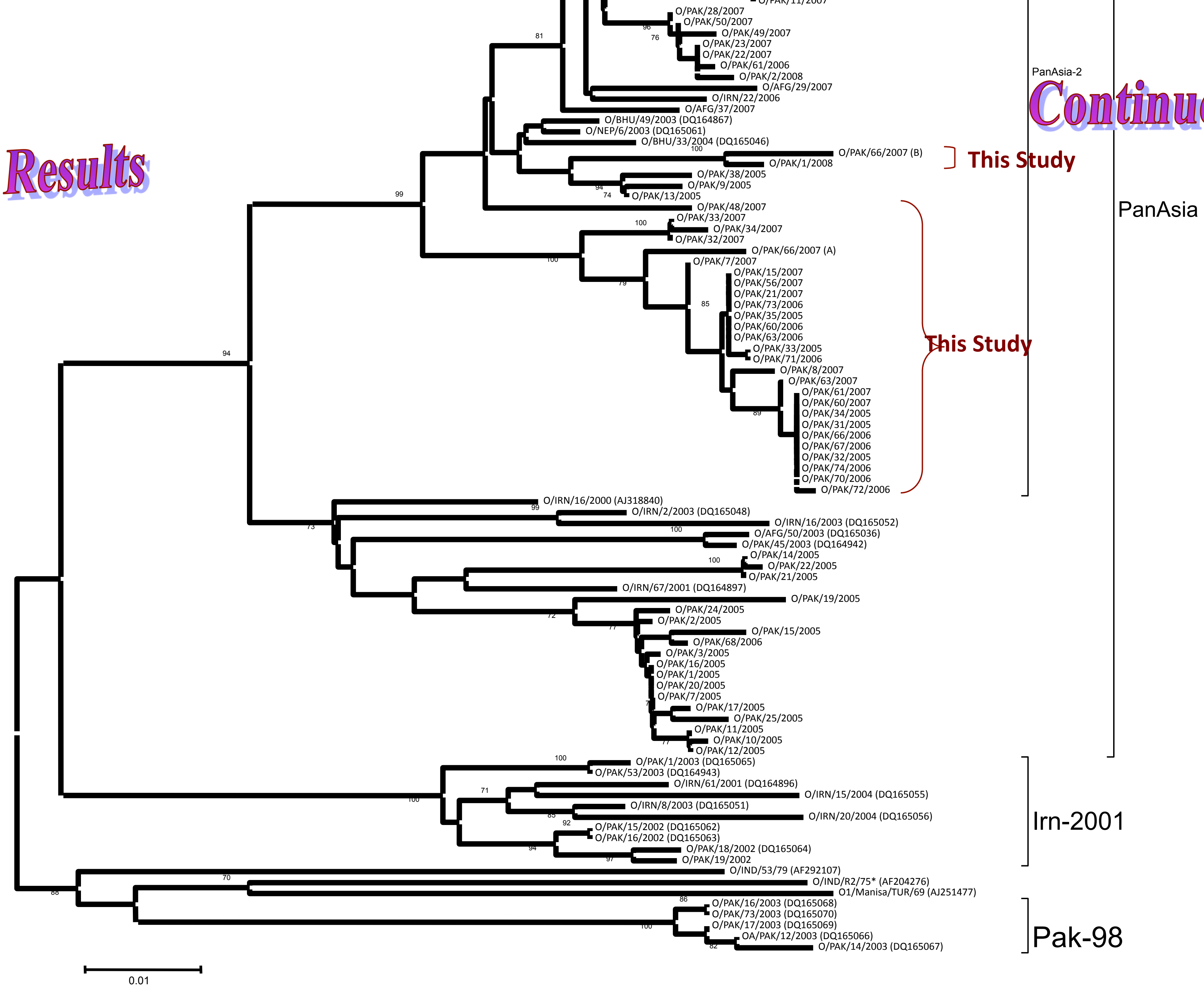
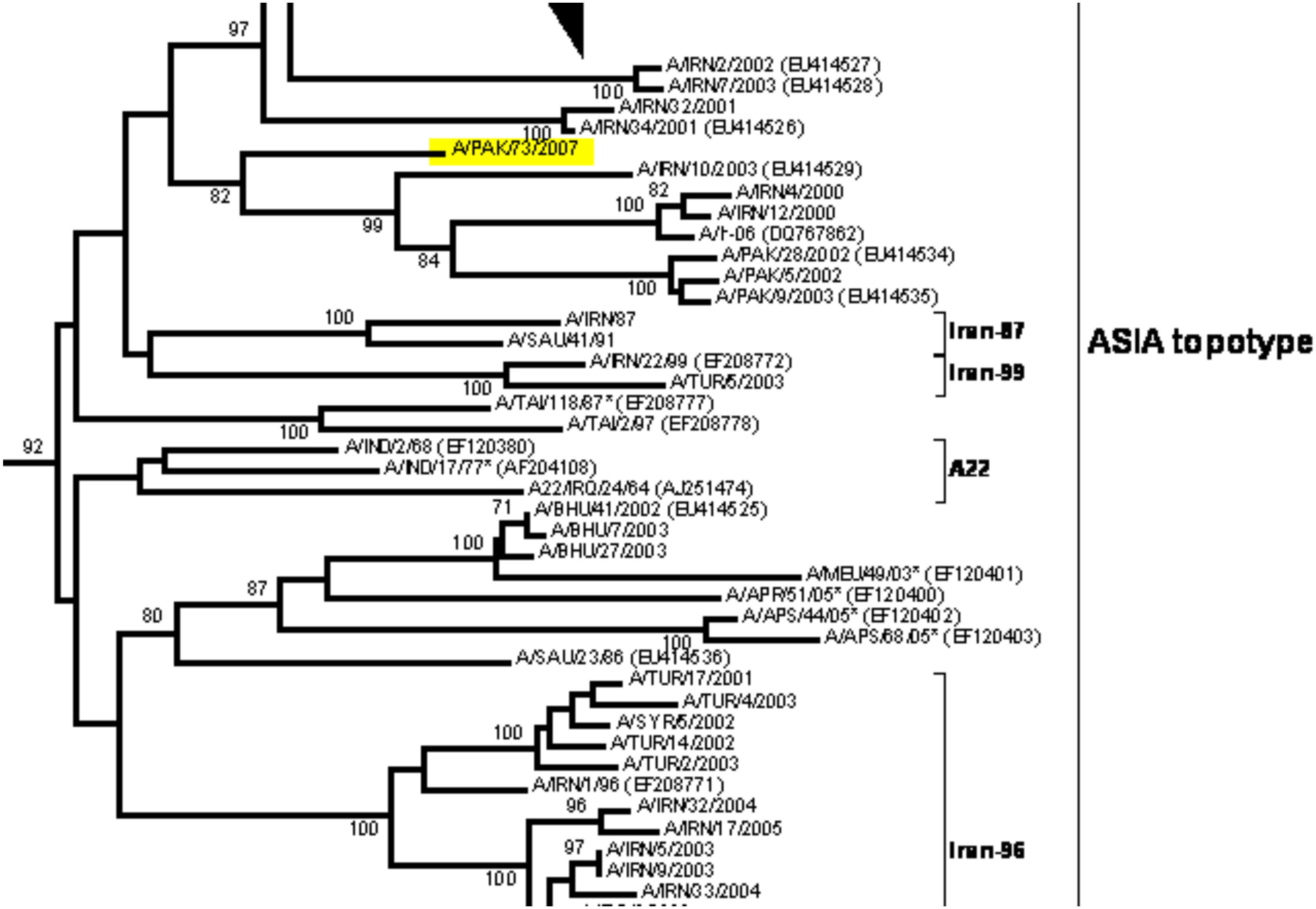


PHYLOGENETIC ANALYSIS OF FMDV ‘A’



Conclusions

- FMD is endemic in the areas of study

- Same FMDV reoccurred in consecutive years during FMD outbreaks with only minor changes in the nucleotide sequence

Acknowledgements

- Nick Knowles, The Pirbright Institute, UK
- Schumann and Xu, PIADC, USDA, USA
- University of Veterinary & Animal Sciences, Lahore, Pakistan
- Funding Agency and GFRA-2015

Thanks for attention!