

# Establishing the role of healthy Asian buffaloes in persistence of foot-and-mouth disease virus

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# Introduction

- ✓ **Vesicular disease** Cloven-hoofed animals & < 70 wildlife species
- ✓ **Etiology** Aphthovirus genus/Picornaviridae (Belsham, 1993)
- ✓ **Economic losses** Entire Asian region (Tamilselvan et al., 2009)
- ✓ **Pakistan** Rs. 6 billion annually (Zulfiqar, 2003)
- ✓ **Nature** Endemic to Pakistan
- ✓ **Serotypes** O, A and Asia-1 (Zahur et al., 2006)
- ✓ **Farmers** Production losses, exports and food security
- ✓ **Persistence** 3-5 years (cattle & African buffaloes)  
(Condy et al., 1985; Thomson et al., 1984)
- ✓ **Silent carriers** Cattle & African buffaloes (McVicar & Suttmoller, 1976)
- ✓ **Recovery** Live virus, 38 dpi (epithelia of pharyngeal region, lymph node in cattle (Juleff et al., 2008)
- ✓ **Earlier work** In cattle and African Buffaloes
- ✓ **Present study** Riverine Buffaloes?

## ➤ **Research questions**

- Does FMDV persists in Asian buffalo
- If yes, can it be detected
- Where is the virus
- Which test is better to detect the virus probang or oral swab
- either virus isolation or PCR

## ➤ **Criteria for persistence/ case definition**

- Non-structural protein (NSP) FMDV positive animals
- Probang positive animals 2X >28 days apart
- No history of outbreak during past 28 days

➤ **Study type:** Prospective study

➤ **Target animals:** Asian Buffaloes

➤ **Year:** 2010-12

## ➤ **Data collected**

- Developed individual animal data collection sheet comprising of following points
- Animal ID (Unique identifier for each animal, Ear tags 1-300)
- Age, Sex, Species, History of FMD vaccination, History of FMD)

## ➤ **Farm selection criteria**

- At least  $\geq 20$  animals
- Animals with or without history of FMD vaccination
- No. of farms which were included in the study: 30 farms in high risk areas

## ➤ **Sampling Criteria**

- Animal with age  $> 6$  months
- 10 samples from each farm, (300 samples together)

➤ **Blood collection**

➤ Sera

➤ **Oro-pharyngeal fluid collection**

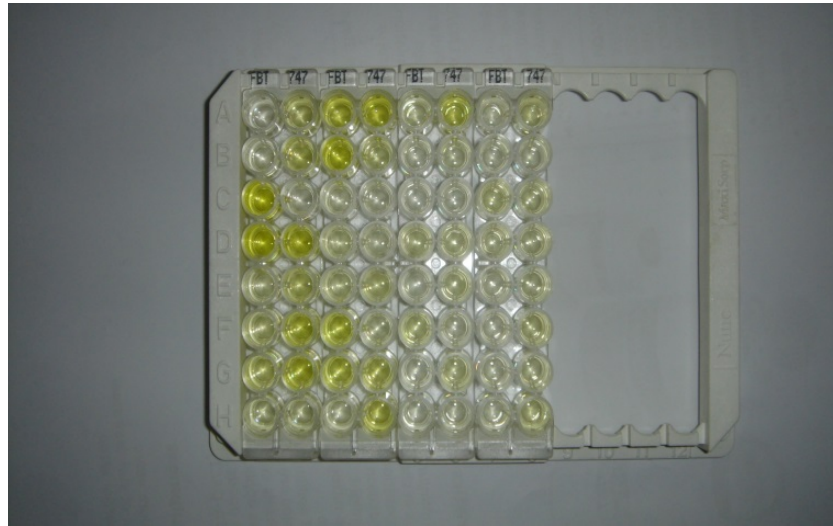
➤ OP fluid collected from donor buffaloes using probang cup (1<sup>st</sup> round)

➤ Subsequent OP fluids collected only from FMDV NSP-Positive animals (for a year at quarterly interval).



➤ **Detection of FMDV specific antibodies in sera**

- CHEKIT FMD-3ABC bo-ov kit, IDEXX Laboratories, USA)



➤ **Detection of FMDV specific genome in OP fluid**

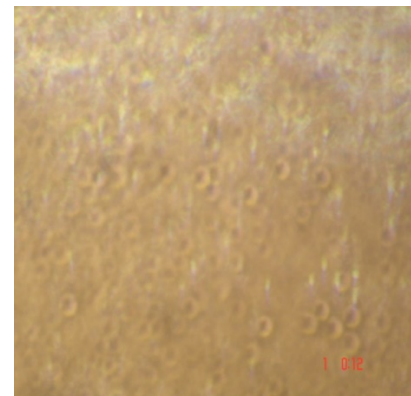
- RNA extraction and real time PCR

➤ **Processing of OP fluid for virus recovery**

- TTE, porcine kidney cell line (LF-BK)

## ➤ Confirmation of FMDV isolate

- CPEs
- RT-PCR
- Antigen detection ELISA (Sero-typing)



## ➤ Result Interpretation

Ct values	Interpretation	Indication
< 29	strong positive	Abundant target nucleic acid in the sample
30-37	positive reactions	Moderate amounts of target nucleic acid
38-40	Weak reactions indicative of minimal amounts of target nucleic acid	Represent an infection state or environmental contamination?

## RESULTS

**89 /300 Animals were determined to be persistent  
(2 or more PCR positive probangs/ no clinical signs)**

**51/89 Animals were found to be positive for VI at  
least once.**

**22/51 Animals were VI positive in 2 or  
more sampling time points**

**29/51 Animals were VI positive only once**



**26/51 Animal VI + sequenced**

**Asia 1 = 18**

**O = 2**

**A = 6**

**TOTAL VP1 = 26**  
**ASIA 1 = 18**  
**O = 2**  
**A = 6**

# Longitudinal Persistence Study

Tag No.	Date of 1st sampling	1				Date of 2nd sampling	2				Date of 3rd sampling	3				Date of 4th sampling	4			
		NARC	PIADC	VI	Seq.		NARC	PIADC	VI	Seq.		NARC	PIADC	VI	Seq.		NARC	PIADC	VI	Seq.
59	10/1/12	31.08	32.54	POS	A	14/3/2012	31.32	35.83			4/6/12	32.10	nd			18/9/2012	nd			
95	18/1/2012	35.92	nd			20/3/2012	37.11	nd			7/6/12	32.18	nd			16/10/2012	nd	nd		
106	24/1/2012	15.72	33.25			22/3/2012	nd	nd			12/6/12	nd	nd			29/10/2012	nd	nd		
112	24/1/2012	39.05	39	POS	ASIA1	22/3/2012	nd	nd			12/6/12	nd	nd			29/10/2012	nd	nd		
131	30/1/2012	nd	nd			26/3/2012	nd	nd			20/6/2012	29.42	35.97			5/11/12	37.56	nd	POS	A
168	7/2/12	26.48	34.84	POS	ASIA1	2/4/12	30.25	36.99	POS	A	3/7/12	36.28	38.73	POS	A	20/11/2012	32.06	40.28		
181	8/2/12	26.54	32.05	POS	ASIA1	3/4/12	30.97	36.88	POS	nd	9/7/12	34.73	36.22			21/11/2012	nd			
184	8/2/12	30.03	34.72	POS	ASIA1	3/4/12	25.23	35.46	POS	ASIA1	9/7/12	29.14	33.12	POS	ASIA1	21/11/2012	nd	nd		
189	9/2/12	nd	nd			4/4/12	27.84	35.24	POS	ASIA1	11/7/12	16.02	35.14			22/11/2012	28.03	36.66	POS	ASIA1
213	16/2/2012	nd	nd			17/4/2012	32.41	41.24			24/7/2012	36.29	nd			28/11/2012	34.96	38.04		
238	22/2/2012	34.24	36.78			19/4/2012	nd	nd			1/8/12	nd	nd			3/12/12	nd	nd		
294	5/3/12	nd	40.11			15/5/2012	nd	nd			29/08/2012	nd	nd			17/12/2012	nd			

## Remarks:

- 12 buffalo – followed for 1 year
- 8 with two of more PCR or VI positive (ie persistent infection)
- 1 case serotype Asia1 substituted by A
- 4/8 animals remaining persistent after one year

## FMDV SEROTYPES ISOLATIONS

	Serotype	1st	2nd	3rd	4th	Total
<b>Animals=51</b> <b>Farms=18</b>	A	9	1	3	3	16
	Asia 1	17	7	7	21	52
	ND	3	1			4
	O	4	1	-	-	5
<b>Total</b>		33	10	10	24	77

# FMDV control program & collaborative partners in Pakistan



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



Animal Health Programme, PARC for research (Dr Khalid Naeem and team)



Team of GCP/PAK/123/USA Project Progressive Control of FMD in Pakistan (Dr. M. Afzal, Dr. Manzoor Hussain, Dr. Ehtisham Khan)



Animal Husbandry Commissioner Office, National Veterinary Labs, Islamabad



THANKS