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 & Sutmoller, 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 >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 (1st 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

<table>
<thead>
<tr>
<th>Ct values</th>
<th>Interpretation</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 29</td>
<td>strong positive</td>
<td>Abundant target nucleic acid in the sample</td>
</tr>
<tr>
<td>30-37</td>
<td>positive reactions</td>
<td>Moderate amounts of target nucleic acid</td>
</tr>
<tr>
<td>38-40</td>
<td>Weak reactions indicative of minimal amounts of target nucleic acid</td>
<td>Represent an infection state or environmental contamination?</td>
</tr>
</tbody>
</table>
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

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

Asia 1 = 18
O = 2
A = 6
### Longitudinal Persistence Study

| Tag No. | Date of 1st sampling | Date of 2nd sampling | Date of 3rd sampling | Date of 4th sampling | 1 | 2 | 3 | 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 | 5/11/2012 | nd POS A |
| 168     | 7/2/2012            | 26.48               | 34.84               | POS                | ASIA1 | 2/4/2012  | 30.25   | 36.99   | 3/7/2012 | 36.28   | 38.73 | 20/11/2012 | 32.06   | 40.28 |
| 181     | 8/2/2012            | 26.54               | 32.05               | POS                | ASIA1 | 3/4/2012  | 30.97   | 36.88   | 9/7/2012 | 34.73   | 36.22 | 21/11/2012 | nd                 |
| 184     | 8/2/2012            | 30.03               | 34.72               | POS                | ASIA1 | 3/4/2012  | 25.23   | 35.46   | 9/7/2012 | 29.14   | 33.12 | 21/11/2012 | nd nd             |
| 189     | 9/2/2012            | nd                  | nd                  |                   | 4/4/2012  | 27.84   | 35.24   | 11/7/2012 | 16.02   | 35.14 | 22/11/2012 | 28.03   | 36.66 |
| 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      | 1/8/2012 | nd      | nd  | 3/12/2012 | nd nd             |
| 294     | 5/3/2012            | 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
<table>
<thead>
<tr>
<th>Serotype</th>
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<th>3rd</th>
<th>4th</th>
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<td>3</td>
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<tr>
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<td>7</td>
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<td>52</td>
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<tr>
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<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>O</td>
<td>4</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>10</td>
<td>10</td>
<td>24</td>
<td>77</td>
</tr>
</tbody>
</table>
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