

SAMPLING, SHIPPING AND TESTING OF FMDV INACTIVATED SAMPLES

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National Centre for Foreign Animal Disease (NCFAD) – Canadian Food Inspection Agency (CFIA)

- NCFAD's mandate is to provide scientific and laboratory services for the rapid and accurate identification and report of foreign animal diseases.
- NCFAD is prepared to handle foreign animal, zoonotic and emerging diseases, with lab and animal space at containment levels 2, 3 and 4
- With a staff complement of ~ 80, NCFAD's areas of expertise includes: virology, molecular biology, pathology, immunology/serology and reagent development
- NCFAD's diagnostic capabilities include e.g. : Classical Swine Fever, Swine Vesicular Disease, Avian Influenza, Foot and Mouth Disease, Vesicular Stomatitis, Bluetongue/EHD, Rinderpest, Capripox and zoonotic/emerging diseases
- NCFAD's scientific program objectives include e.g. : development and production of quality reagents and updated methods; expand our response capability to an FAD, zoonotic disease or emerging disease outbreak, response to bioterrorism and training in FAD diagnostics.

Collaborative project on the Epidemiology of FMD in Pakistan started in January 2006 – Focus on Landhi Dairy Colony



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Manzoor Hussain
Mohammad Munir
Giancarlo Ferrari
Keith Sumption

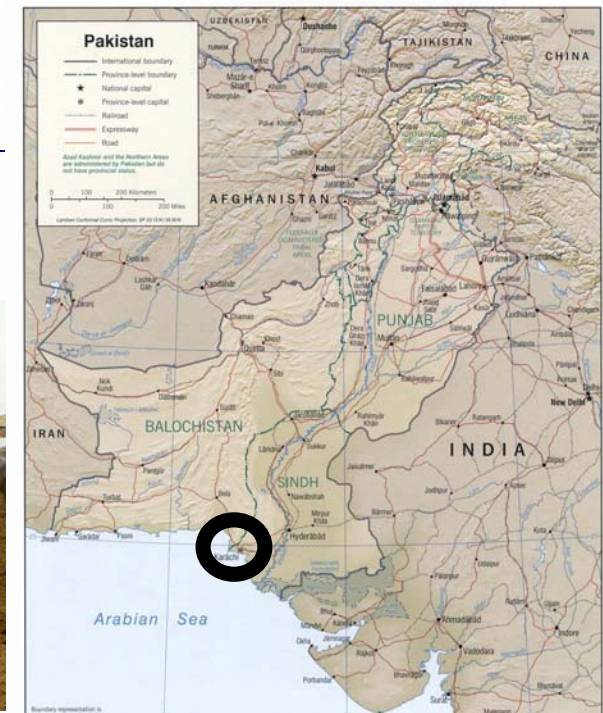


Muhammad Afzal
and the many helpful
veterinarians in Pakistan →



David Paton

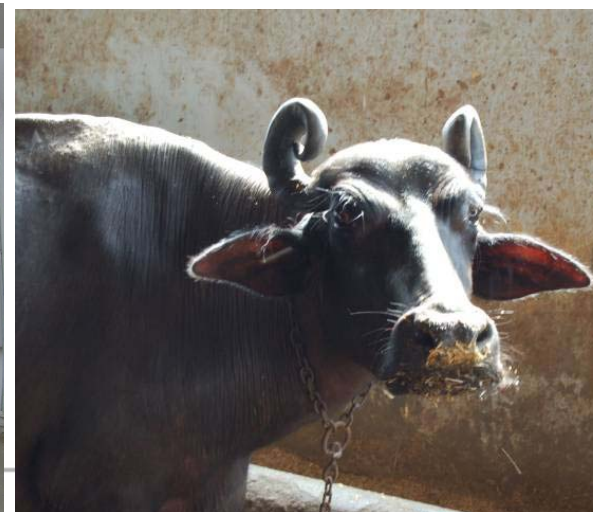
Abdul **Hafeez** Shaikh
Mohammad Hassan Tanveer
Mehmood Iqbal Khan
Manzoor **Asif**
Zakaullah Nizamani
Mohammad Adil Yakoob
Syed **Noman**
Atiq-ur-Rheman
Iacat Ali





Goals: To describe the epidemiological situation of FMD in Pakistan's largest dairy colony at Landhi

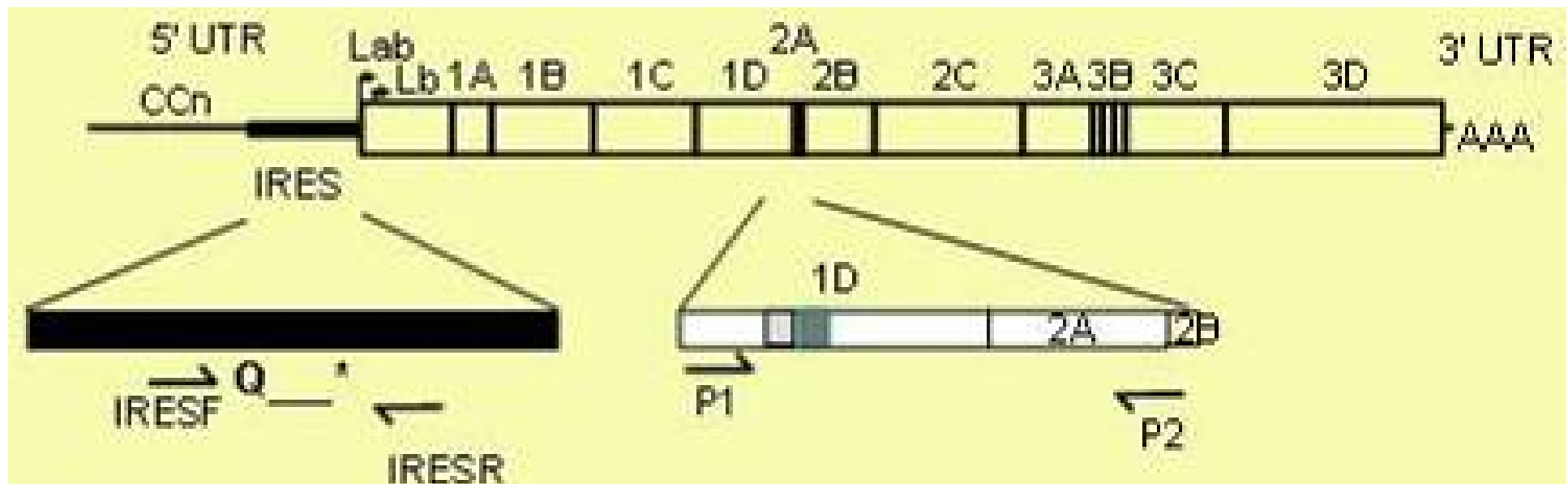
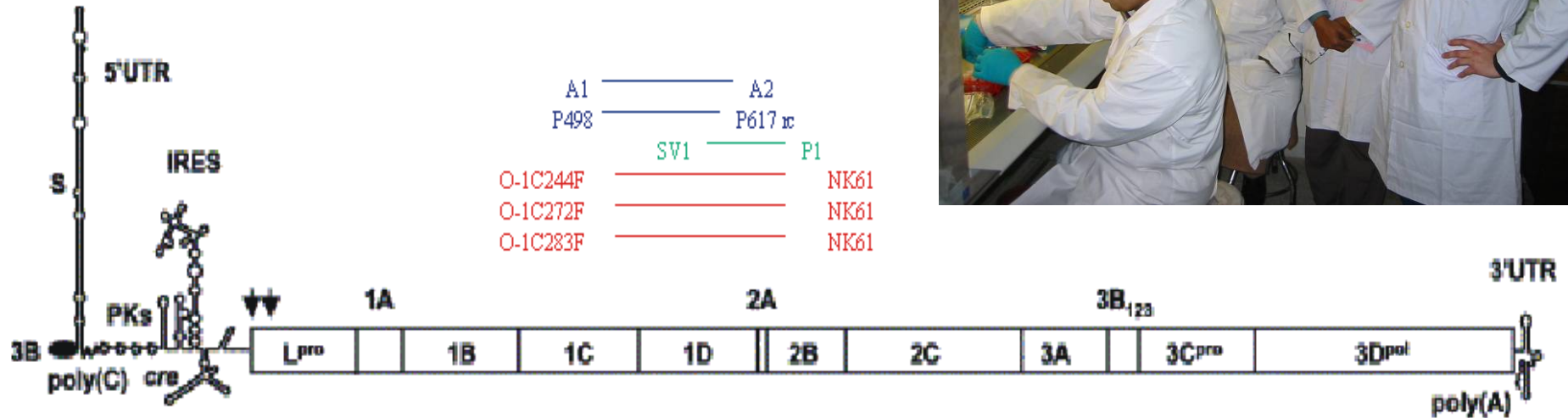
**Started in January 2006
Made 5 visits + 9 monthly samplings, collected ~1500 swab samples, 150 serum samples & ~ 50 epithelium samples**



In the laboratory: RNA extraction, making cDNA



Back in Denmark: **Real time RT-PCR and sequencing**



Sampling scheme

Large samplings: April & September 2006 and January and April 2007:

18 farms, taken mouth swabs from ~ 9 buffalo and 1 cow

Smaller monthly samplings: 5 farms taken mouth swabs from ~5 buffalo and 1 cow

Mouth swabs were immediately placed into 1.0 ml of RLT lysis buffer (Qiagen) in 2 ml screw-cap polypropylene tubes (Sarstedt). Such swabs in lysis buffer were often kept at ambient temperature for many hours before being stored at -20°C.

Then shipped directly to Denmark, or alternatively via the National Reference Laboratory in Islamabad for RNA extraction followed by cDNA preparation and shipping of prepared cDNAs to Denmark.

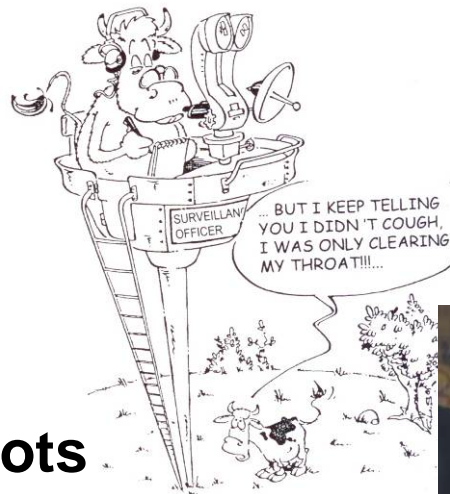
Swabs in lysis buffer and prepared cDNAs contain no infectious FMDV and can relatively easily be shipped as diagnostic samples both locally and internationally.

Group A: Randomly selected, non-clinically affected animals from non-affected herds

Group B: Samples from non-clinically affected animals in herds with the presence of a few other animals with evidence of old and healing lesions

Group C: Samples from non-clinically affected animals in herds in which acute FMD was obvious in at least one other animal. Lesion epithelia samples were in addition also collected during a pre-study visit in Jan/Feb 2006





Help in getting truly random samples

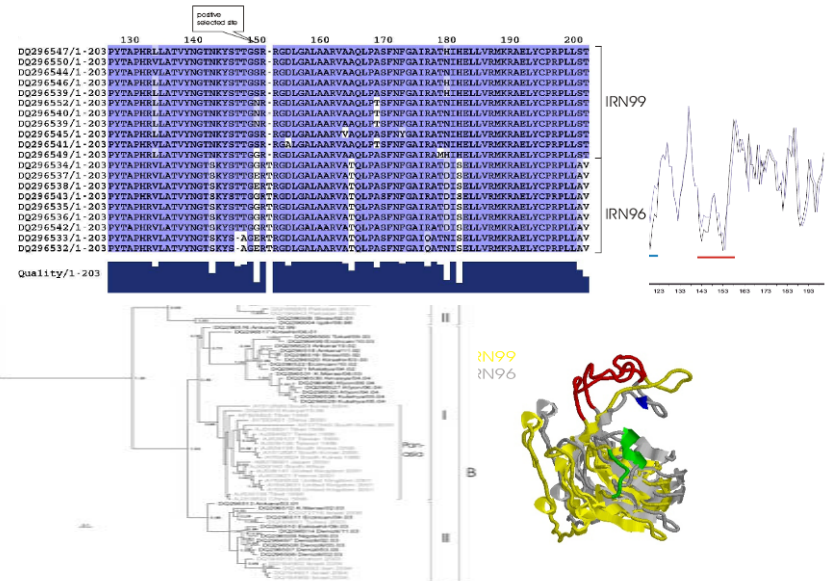
A combination of

“shoe-leather/rubber boots epidemiology”



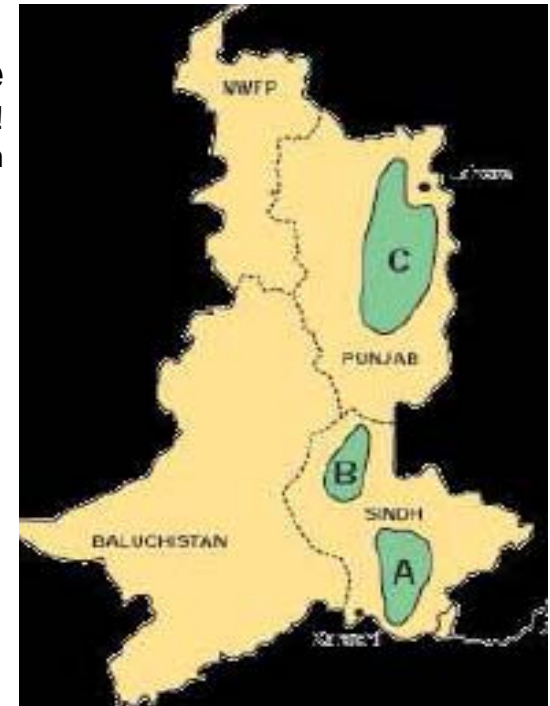
and

“armchair and molecular epidemiology”



1. Market Structure

Animals are kept for only one lactation period!
=> 10-12% month



2. Religious festival – Eid ul-Azza



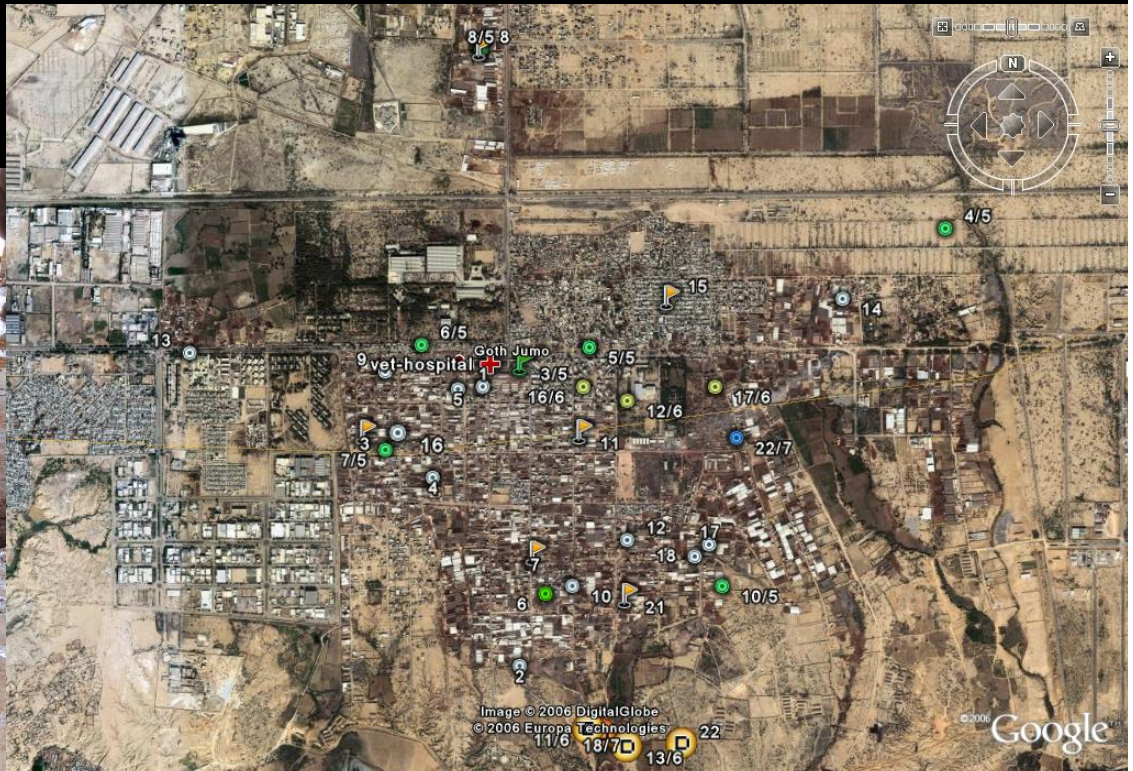
3. Vaccination strategy

The majority of commercial dairy farmers use FMD vaccine

Usual practise vaccinate once with e.g. Aftovax and/or several times with monovalent-(O)- vaccine produced in Lahore.

Also vaccines imported from India, China, former Yugoslavia and Spain etc.

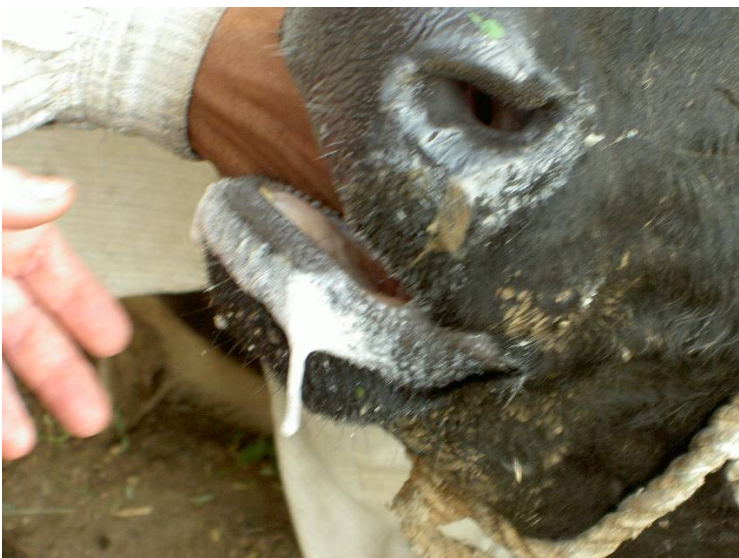


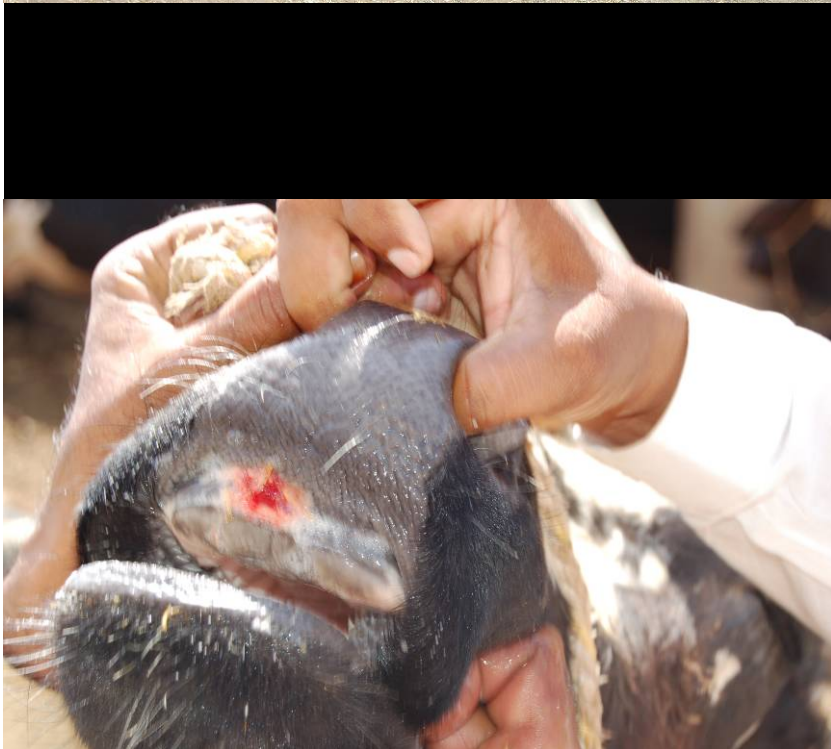


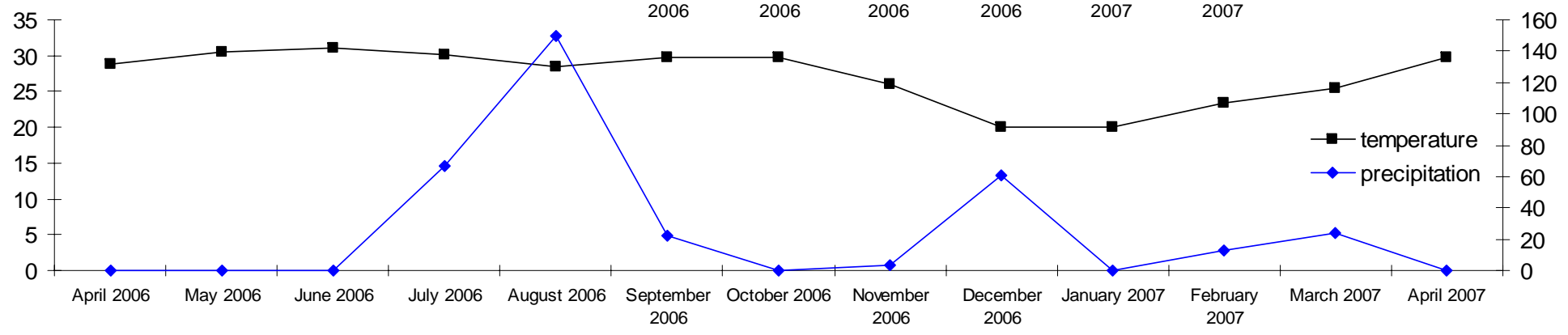
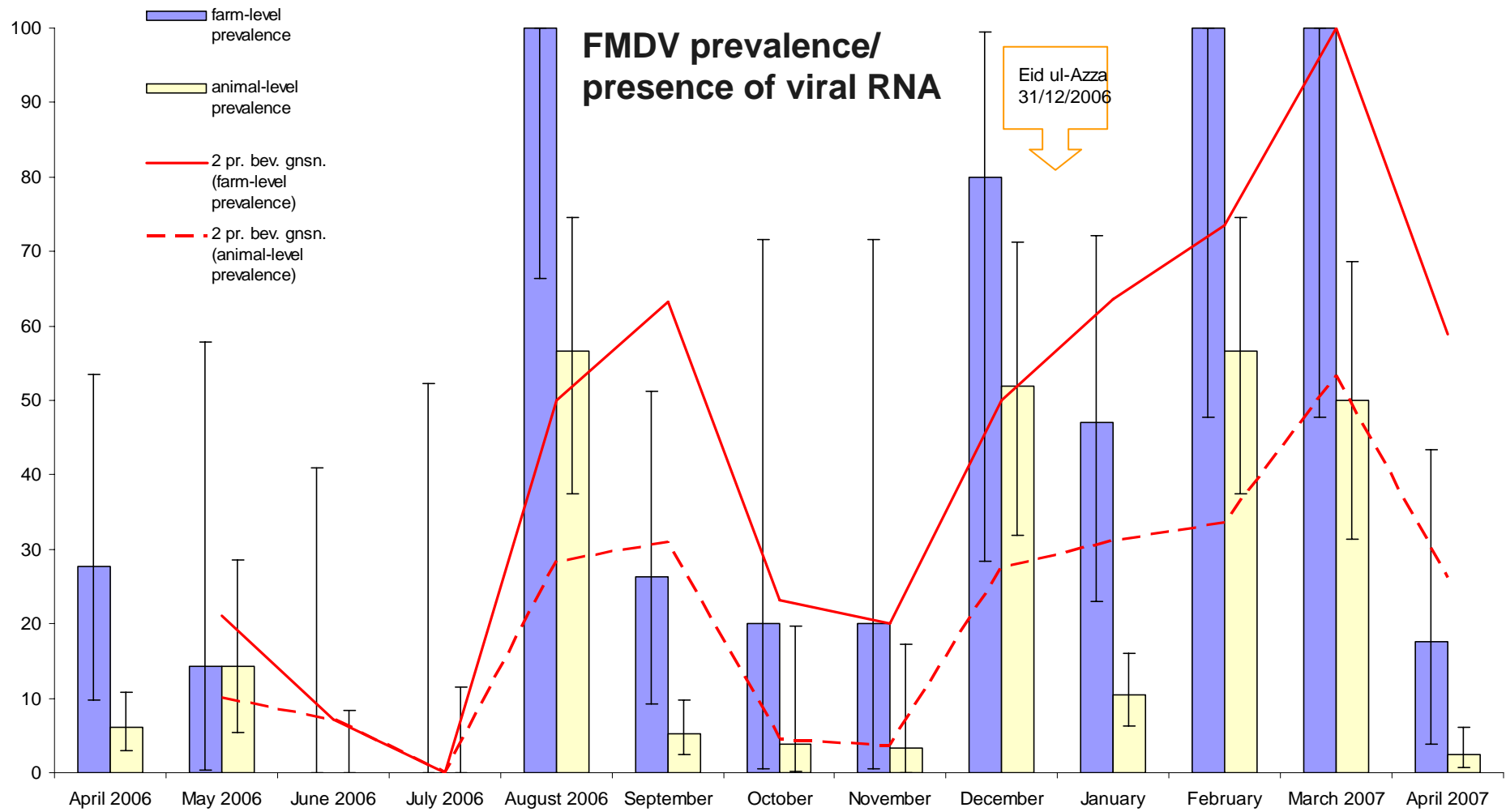




FMD – High yielding Buffalo and cattle in Pakistan







RESULTS REAL TIME RT-PCR

FMDV RNA Detected in Swab samples during the 1 year study (1034 swabs analysed)

Group A: Randomly selected, non-clinically affected animals from non-affected herds
960 mouth swabs from 124 farms – 106 swabs positive for FMDV RNA (**11%**)
58 sequenced (partial or full VP1) (55%)

Mean Ct of 36.0 (range 26 to 39) ~ 10^{4.6} copies/ml (range 10^{3.7-7.5})
~ 10² TCID₅₀/ml (range 10¹⁻⁵) (BTY)

(Non-sequenced swabs
mean Ct of 42.5 (range 40-48) ~ 10^{2.7} copies/ml (range 10^{1.0-3.4})
~ 10¹ TCID₅₀/ml (range 10^{0-1.5})

Group B: Samples from non-clinically affected animals in herds with old/healing lesions
45 samples from 4 herds – 22 positive (**49%**)

Mean Ct of 37.5 (range 25-46) ~ 10^{4.1} copies/ml (range 10^{1.6-7.8})
~ 10^{1.5} TCID₅₀/ml (range 10⁰⁻⁵)

Group C: Samples from non-clinically affected animals in herds with acute FMD
29 samples from 2 herds – 25 positive (**86%**)

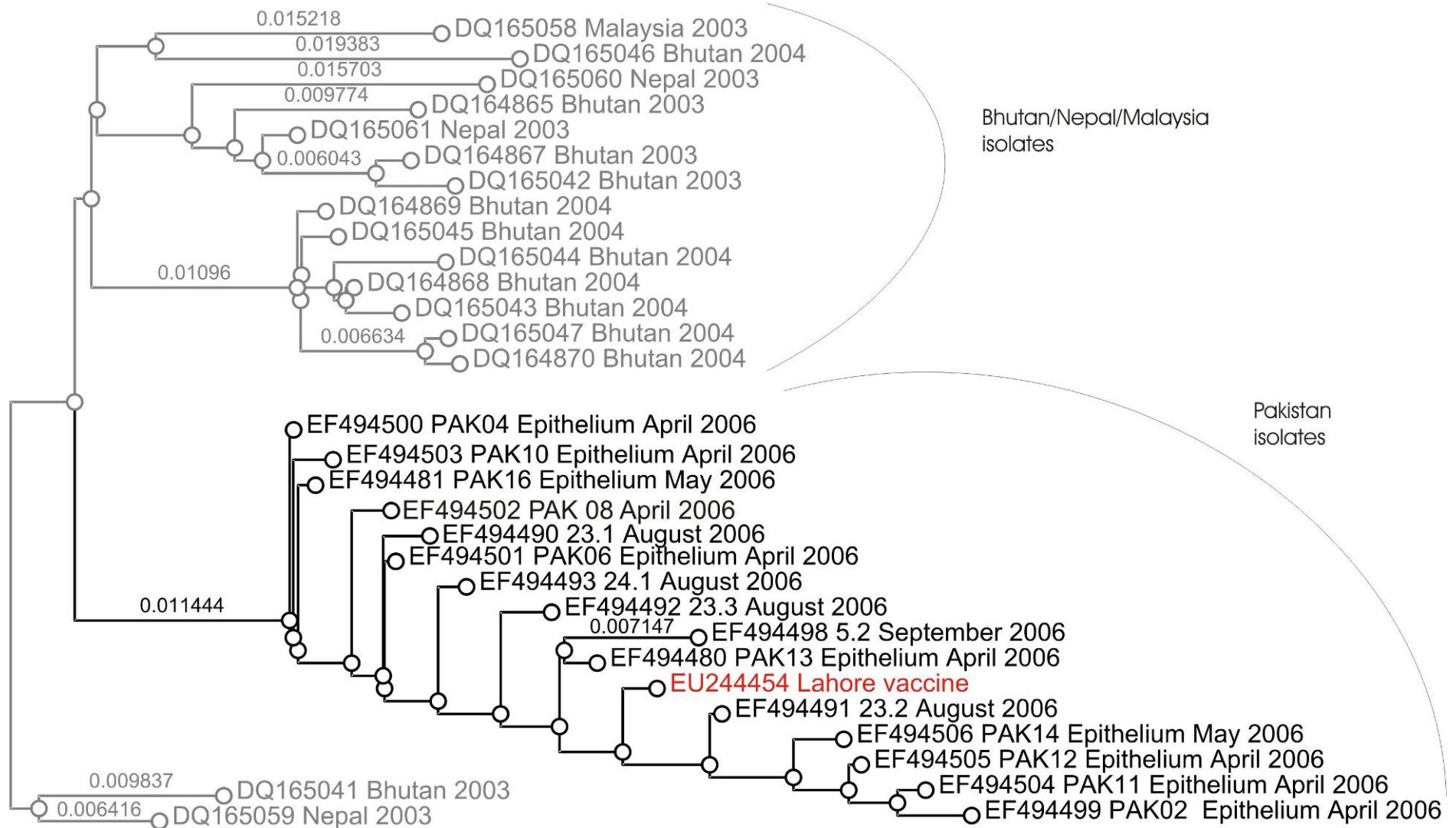
Mean Ct of 32.0 (range 24-39) ~ 10^{5.8} copies/ml (range 10^{3.7-8.1})
~ 10³ TCID₅₀/ml (range 10^{1-5.5})

Prevalence estimations on basis of real time RT-PCR on mouth swab samples

An average point prevalence of ~11% of animals being positive for FMDV RNA in mouth swabs (but without any clinical signs), assuming that an individual infected animal may have **viral RNA for around 14 days and that every animal is replaced each year**, would correspond to an accumulated FMDV infection rate in such herds of 11% x 52 weeks / 2 weeks, i.e. around **200-300% or each animal infected 2-3 times per year.**

Point prevalence in farms having **animals with old FMD lesions** is **~50%** and even **higher** in farms experiencing **acute FMD** (FMDV RNA positive mouth swabs of **~85%**) indicating that **all/most animals in the herd is exposed to FMDV within a period of ~3-4 weeks.**

Bayesian phylogenetic analysis of the full 1D (VP1) nucleotide sequence of Pakistan serotype O isolates (black) and closely related published sequences (grey). The locally produced monovalent vaccine (Lahore vaccine) is red



Conclusions

- Combined field and molecular epidemiological studies using mouth swabs collected directly into a suitable lysis buffer provide a powerful tool for generating detailed epidemiological information and molecular characterisation of circulating strains.
- Direct lysis of collected swab samples into lysis buffer circumvents the need for international shipment of samples as dangerous goods and provides inactivated and stable samples that without any safety problems can be shipped as diagnostic samples.
- Analysis of high numbers of mouth swabs by sensitive, quantitative real-time RT-PCR indicates that this method may provide an efficient way of establishing the extent of virus circulation and for providing estimates of prevalence of infection. Subsequent sequencing can be used for detailed analysis. Extracted RNA may possibly be used for making infectious FMDV by transfection into suitable cell cultures; however, this aspect needs further study.
- Collaborative studies using inactivated and stabilised samples and involving the relevant authorities and laboratories may provide an efficient avenue for strengthening FMD control programs.
- Further studies should be supported to further establish the suitability of such methods.

East Africa



Dubai



Acknowledgement

I thank the many friendly and competent Veterinary Officers as well as Drs. Giancarlo Ferrari and Keith Sumption and the FAO Regional Project and the EU EPIZONE project

Thanks for listening!



Canada 