The application of pooled milk for foot-and-mouth disease surveillance.

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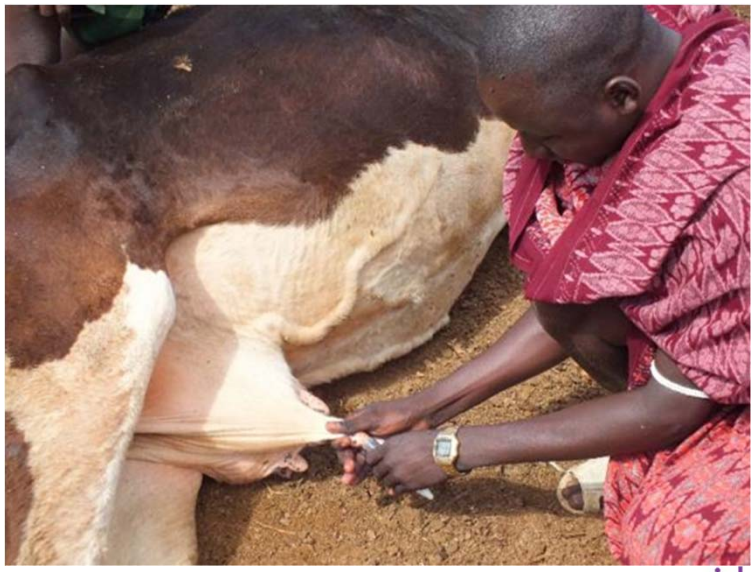
Reduction of sample bias: Milk?

- Diagnostic samples (epithelial tissue) are invasive to collect – also collected from animals with clinical disease.
- Recognition of clinical cases requires farmer engagement and effective veterinary infrastructure
- Furthermore, FMD circulating sub-clinically may not be represented.
- Milk could be a solution in reducing sample bias;
  - Routinely collected;
  - Suitable for FMDV detection and characterisation;
  - Suitable for Ab detection.

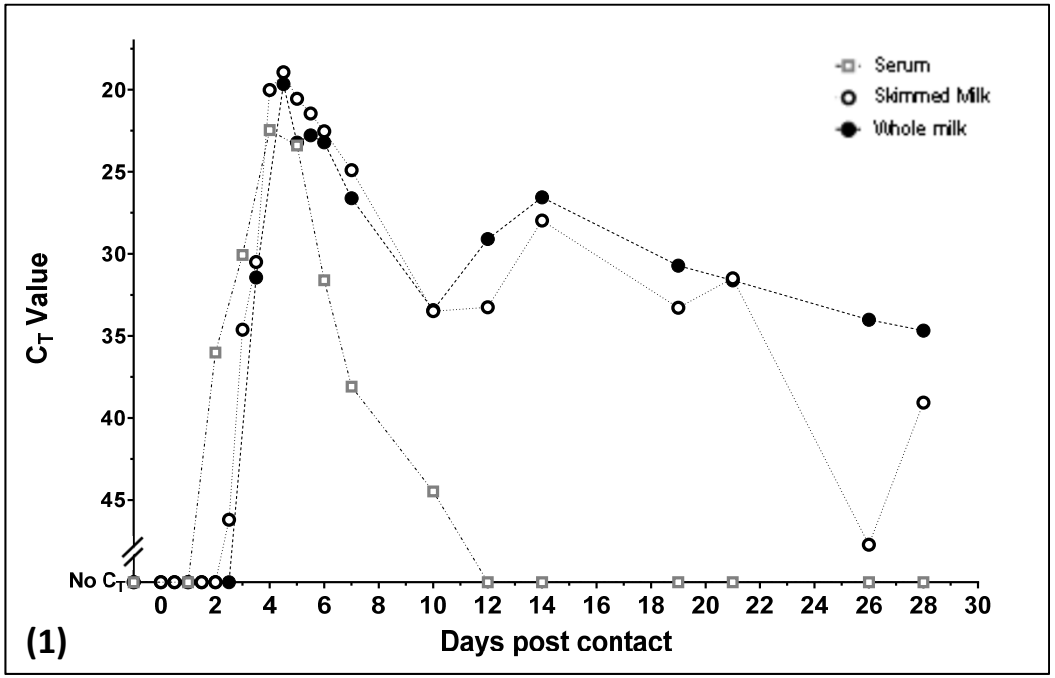


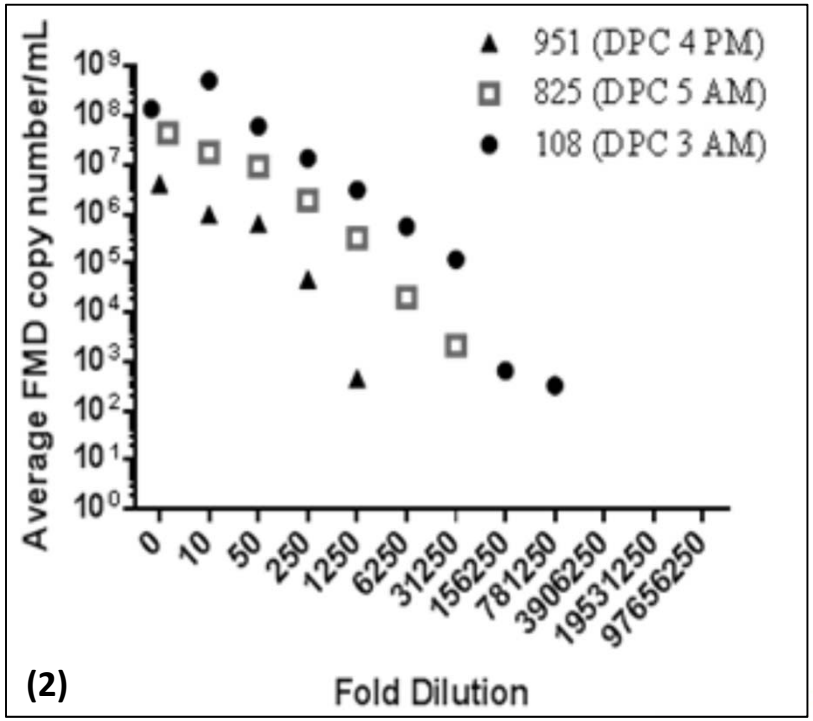
What do we already know?

Experimental studies:

- FMDV can be detected by rRT-PCR in milk in the preclinical, clinical and recovery stages of disease, up to 28 days post contact
  - up to 16 days more than from serum (graph 1).
- It has been shown that FMDV can be detected in milk diluted to over 750,000 fold (graph 2).

Key question: Can **pooled milk** be useful for FMD surveillance in an endemic setting?





Armson *et al.* (unpublished), Reid *et al.,* 2006

Objectives:

Laboratory validation already carried out to optimise analytical sensitivity – two automated extraction and rRT-PCR assays

Testing of pooled milk samples from endemic settings:

- 1206 pooled milk samples from two large scale farms in Saudi Arabia
  - to further evaluate two pan-serotypic 3D rRT-PCR assays*
    (TaqMAN Fast kit and Superscript One-step kit)

Further analysis:

- Compare C_T values in different groups of cattle – where disease was and was not observed.

*Callahan et al. 2002

Study site – Large scale dairy farm in Saudi Arabia

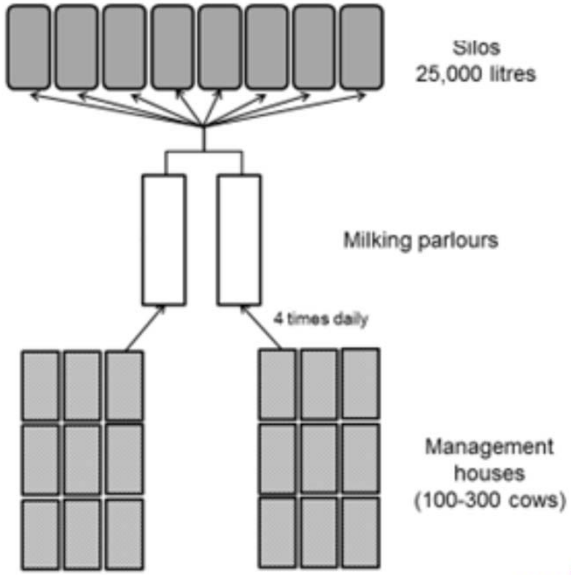
- Cows vaccinated with a hexavalent NSP purified FMD vaccine

  (containing O Manisa, O-3039, A Iran-05, A Saudi-95, Asia-1 Shamir and SAT-2 virus strains) (≥6.0PD_{50})

- Lactating cattle - grouped into management houses (sizes 100-300 cows)
- Two outbreaks occurred - A/ASIA/GVII (September 2015)

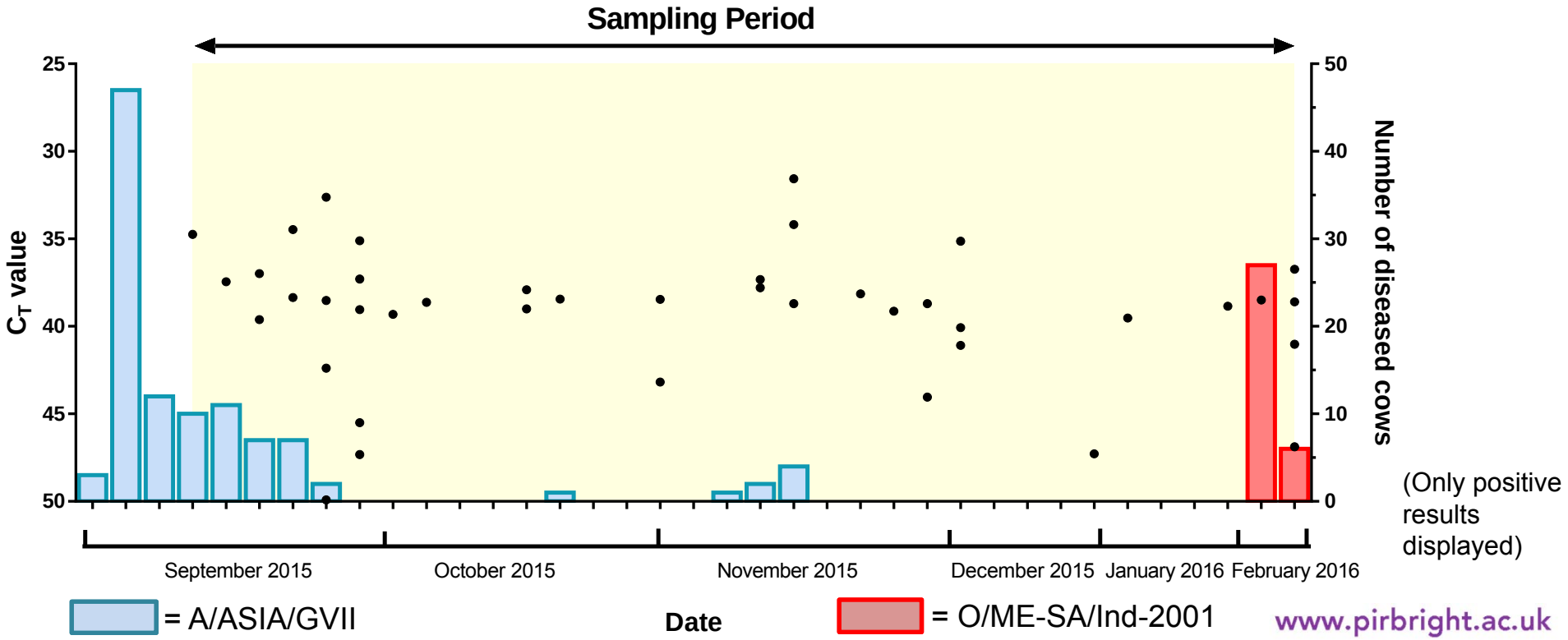
  - O/ME-SA/Ind-2001 (February 2016)

- Milk samples collected twice a week from each management house, using a proportional in line milk sampler.

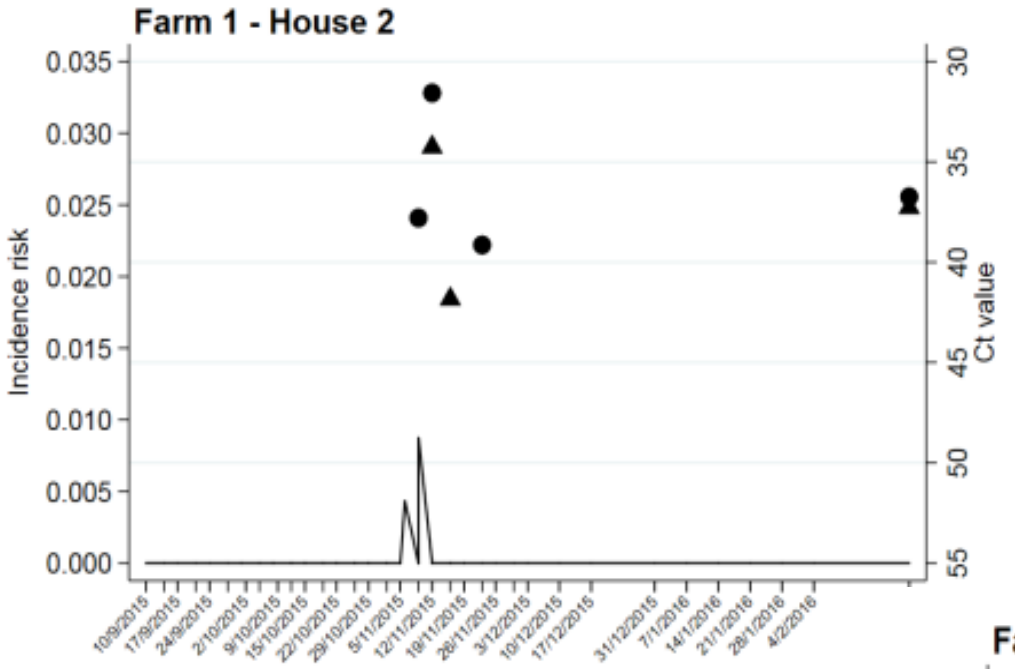


rRT-PCR Results and Preliminary Analysis

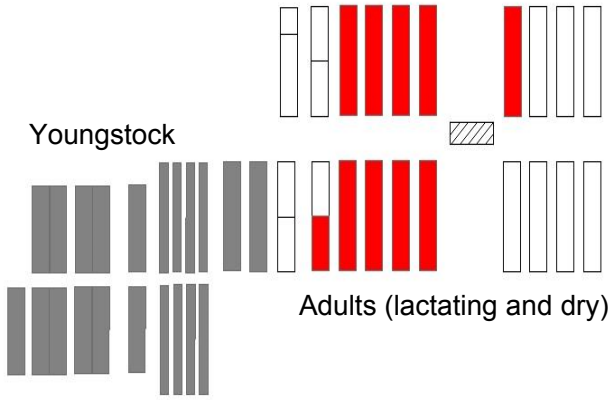
	A outbreak	O outbreak
Number of lactating groups affected	10 / 22 (45.5%)	4 / 23 (17.4%)
Number of clinical cases of FMD	107 / 4000	33 / 4000
Overall incidence risk (number of cases/total livestock on farm)	**2.7%**	**0.8%**
Number of milk samples collected and tested	732	
Positive samples on Superscript rRT-PCR (C_T ≤50)	**42 (5.7%)**	
Positive samples on TaqMan rRT-PCR (CT ≤45)	**19 (2.6%)**	



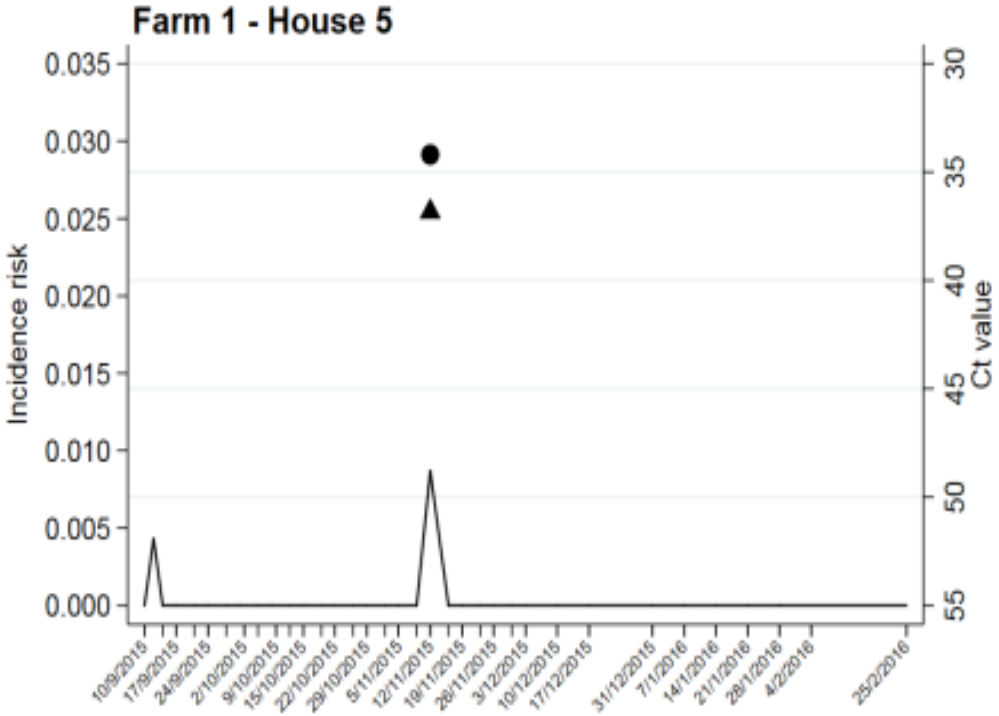
(Only positive results displayed)



Incidence ● Superscript assay ▲ TaqMan Fast assay



- 14/23 houses – clinical disease
- All 14 houses had at least one positive PCR result
- Four houses were positive by rRT-PCR - but had no reported clinical cases



Conclusions

Pooled milk has the potential to be used as a cost-effective, non-invasive, practical surveillance tool.

- FMDV genome was able to be detected from pooled milk samples from up to 240 vaccinated cows
- C_T values were low - likely due to dilution factor

Further work

- Further investigate reasons for instances where rRT-PCR results do not match clinical disease reports, e.g. animal movements, virus spill-over, subclinical infection.
- Use lineage specific rRT-PCR assays (A/ASIA/GVII & O/ME-SA/Ind-2001).
- Model expected C_T results (based on experimental virus profiles during infection) in pooled milk and compare with actual rRT-PCR results taking into account:
  - animal movements
  - milk yields
  - vaccinated animals

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