

A novel BVDV-1 subgenotype detected in a closed beef herd in California 2005



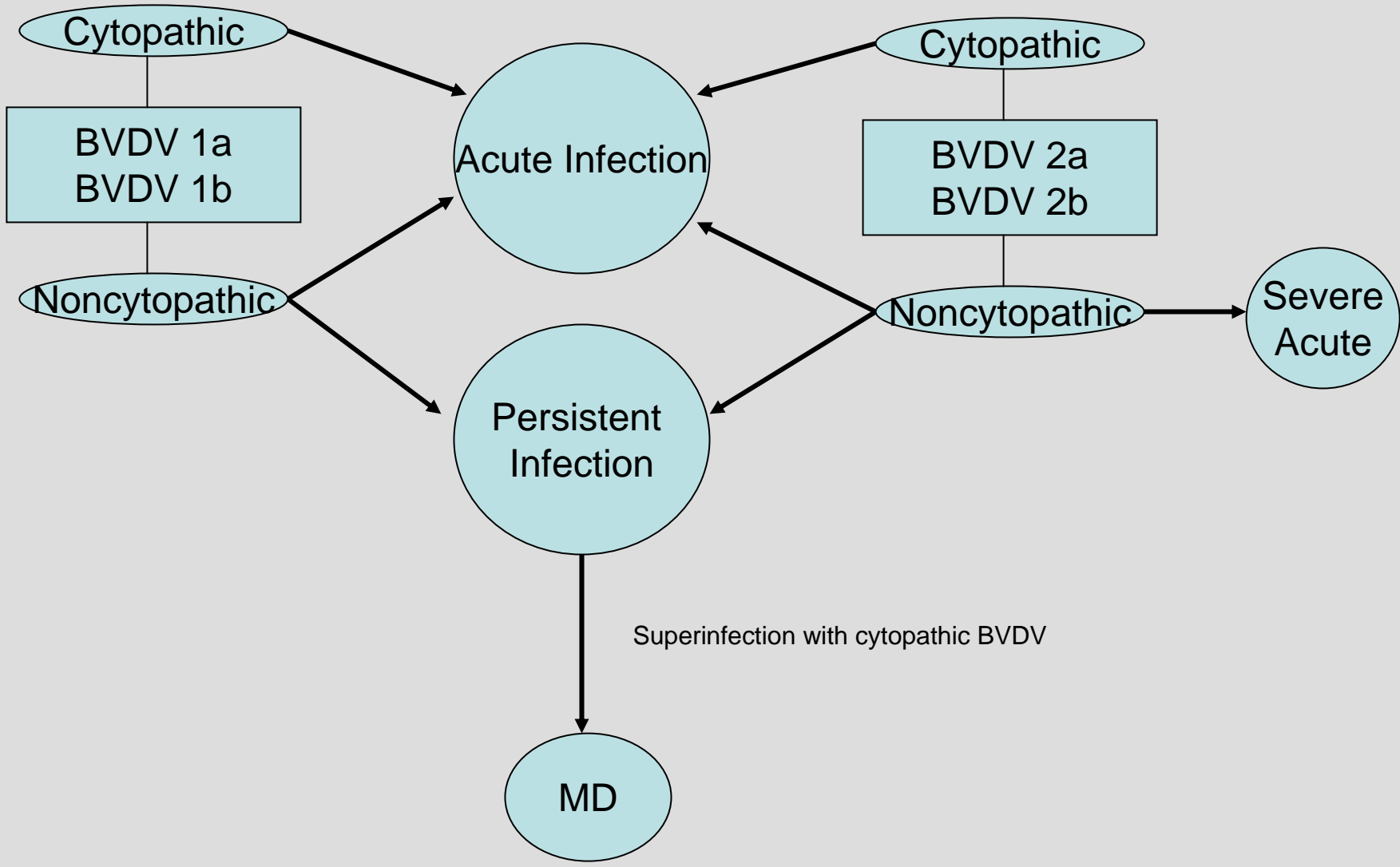
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Pam Hullinger, Sharon Hietala

Quasispecies

$$n_i' = \sum_j w_{ij} n_j \quad , \text{ where}$$

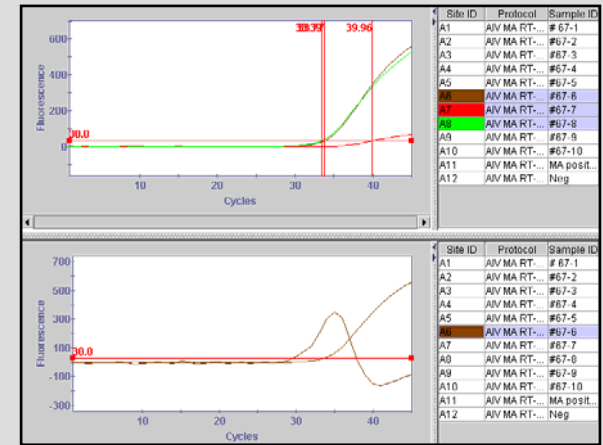
$$w_{ij} = A_i q_{ij} \quad , \text{ and} \quad \sum_j q_{ij} = 1$$

M. Eigen and P. Schuster, *The Hypercycle: A Principle of Natural Self-Organization* (Berlin: Springer, 1979).



CAHFS' BVDV Diagnostic

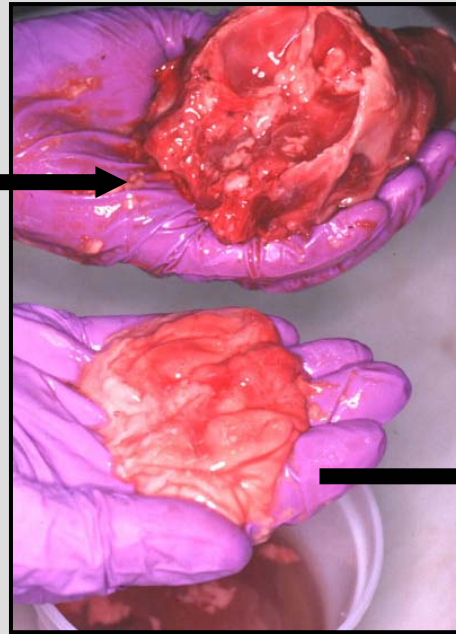
PCR



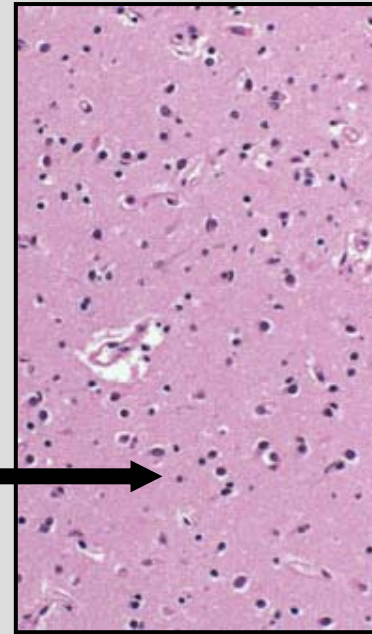
Necropsy



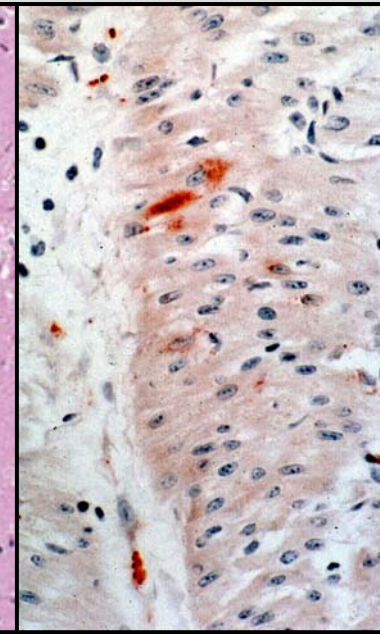
Pathology



Histology



Immuno
histochemistry



Advantages of qRT PCR

1. Sensitivity
2. Specificity
3. Fast TAT



CAHFS' qRT PCR BVDV:

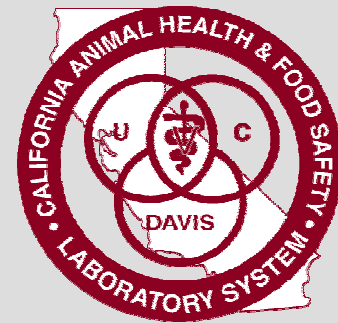
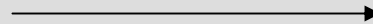
- **Primer** (Virology 1994. 205:66-74)
- **Probe** (CAHFS, 2000)
- **Assay control system**
- **Electrophoresis gel**

BVDV proficiency panel

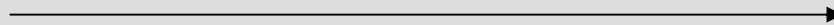
- 4 laboratories
- Volunteer base
- 16 samples
 - 12 blood samples
 - 4 tissue samples
- European style
 - Different extraction method
 - Different primer/probe
 - Different protocol
 - Std PCR/real time PCR

	Lab A	Lab B	Lab C	Lab D
EDTA#1	positive	positive	positive	positive
EDTA#2	negative	negative	negative	negative
EDTA#3	positive	positive	positive	positive
EDTA#4	positive	positive	positive	positive
EDTA#5	positive	positive	positive	positive
EDTA#6	negative	negative	negative	negative
EDTA#7	positive	positive	positive	positive
EDTA#8	positive	positive	positive	positive
EDTA#9	positive	positive	positive	positive
EDTA#10	positive	positive	positive	positive
EDTA#11	negative	negative	negative	negative
EDTA#12	positive	positive	positive	positive
tissue#1	negative	negative	negative	negative
tissue#2	negative	negative	negative	negative
tissue#3	positive	positive	positive	positive
tissue#4	positive	positive	positive	positive

History



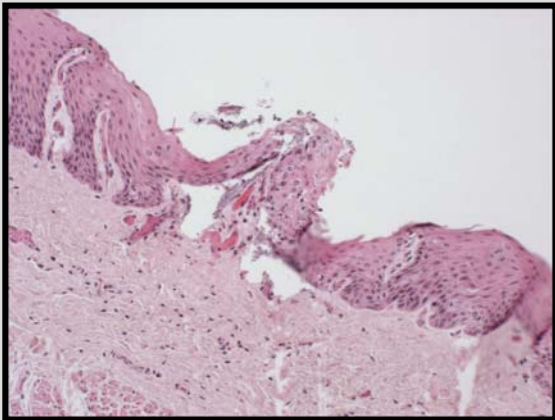
Sunday



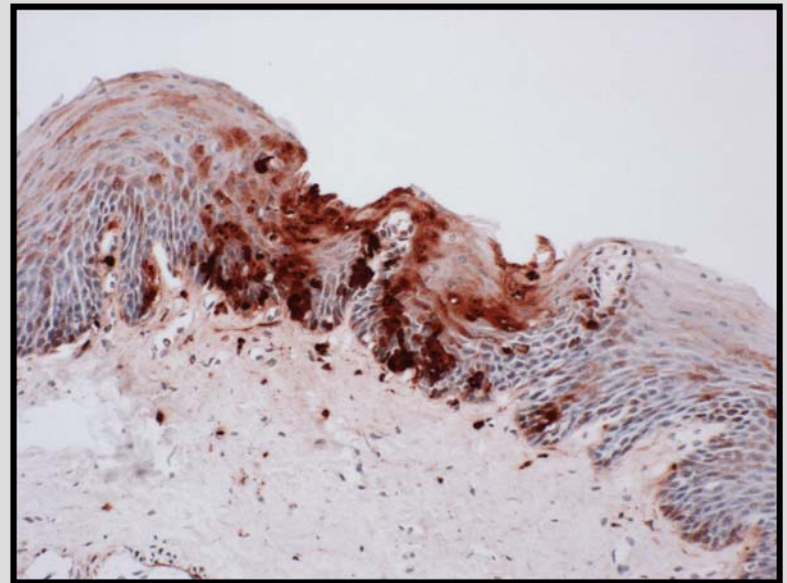
Wednesday

Pathology

- Mild multifocal epithelial necrosis and ulceration in
 - esophagus
 - abomasal mucosa
 - Intestine



- FA: neg/weak
 - BioResearch, polyclonal 1:30
- Immunohistochemistry
 - MAB 15.c.5, Dr Amy Glase, 1:20



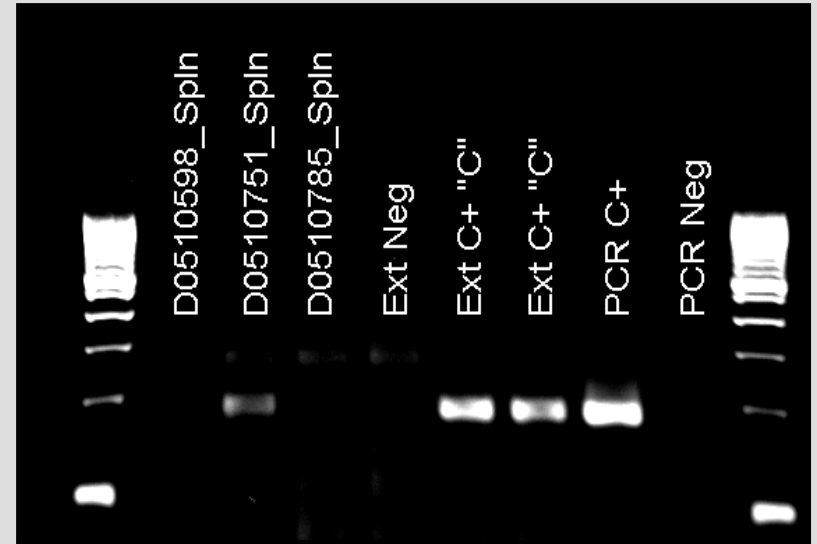
RT PCR results (spleen tissue)

qRT PCR:

negative

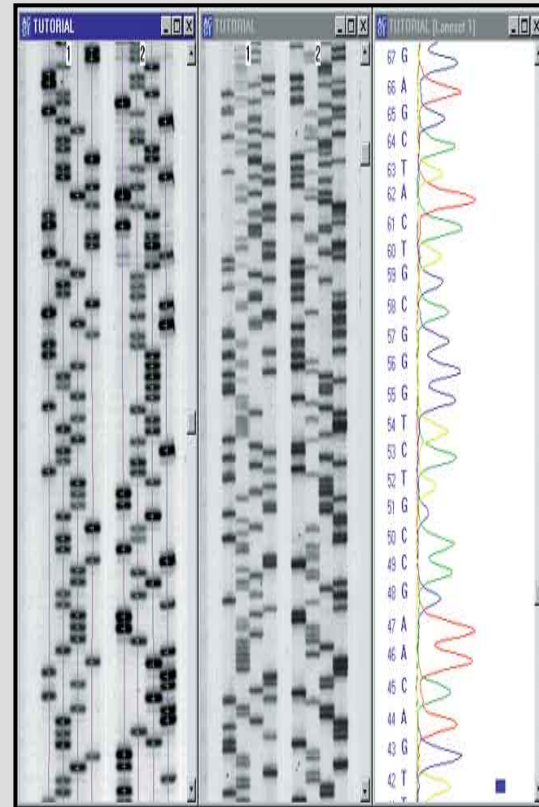
Electrophoresis:

positive



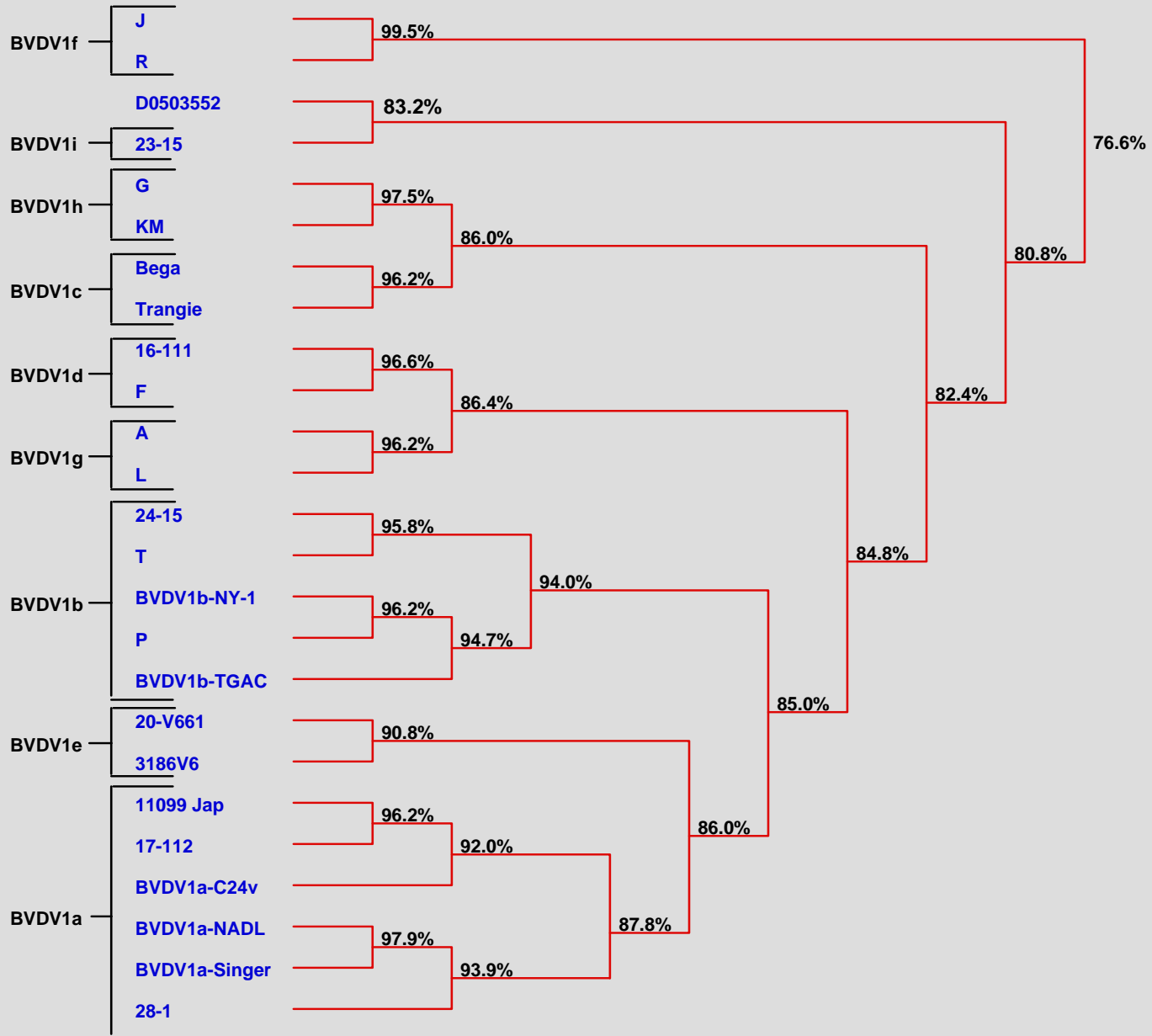
BVDV Sequencing

- BVDV-1 sequence
- GenBank
 - Japanese Isolate
 - South African Isolates grouped as BVDV-1c
- 28 bp deletion
- 2 mismatches in probe binding region

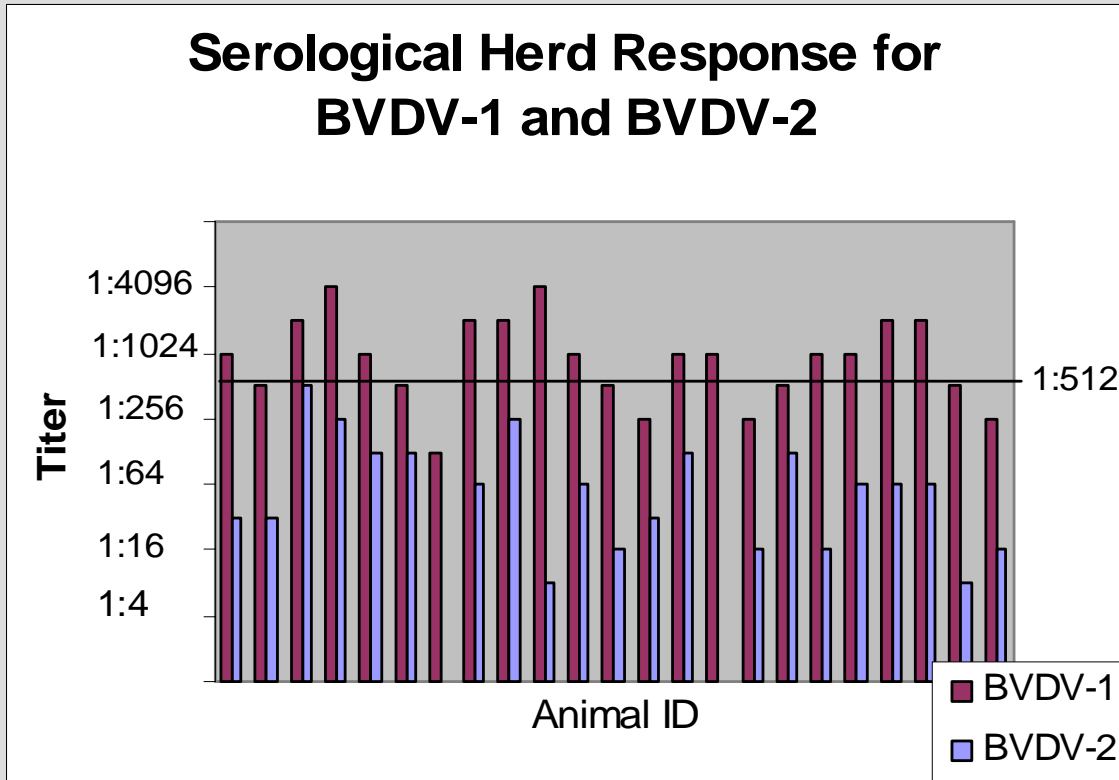


Country	BVDV1 subgenotypes present
Austria	b, d, f, g, h
Germany	a, b, c, d, f, g
Italy	b, d, e, f, h
UK	a, b, i
Slovenia	b, d, f, g
Spain	b, c, h
India	b
Australia	c
U.S.A.	a, b

BVDV1 subgenotypes based on comparison of 5' UTR



Epidemiological Follow-up:



Conclusions

1. New BVDV-1 subgenotype found
 - Epidemiological follow up
 - Spontaneous event?
2. Be cautious with a diagnostic assay for RNA viruses
 - Agarose gel
 - Sequence Analysis



Acknowledgements:

- Participants of the BVDV inter-laboratory proficiency panel
- USDA ARS NADC Pestivirus Research Unit
- CAHFS technical staff
 - Biotechnology
 - Pathology
 - Toxicology