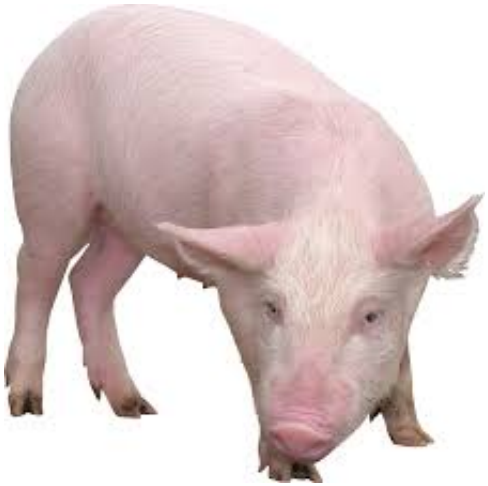
Evaluation of oral swabs for surveillance and high throughput testing for FMDV.

R. J. Davis and P.D. Kirkland, Virology Laboratory,

Elizabeth Macarthur Agricultural Institute (EMAI)

FMDV surveillance options

- Clinical examinations of cattle and pigs are usually considered adequate to provide a high level of sensitivity for the detection of infection in unvaccinated animals.



FMDV surveillance options

- Sheep can often become infected but only show mild or no clinical signs
- Virus loads are lower than cattle or pigs but can act as a reservoir to disseminate virus (eg UK)
- FMDV detection in sheep typically depends on serology or virus isolation on tissues or blood.

FMDV transmission studies

- Over the last decade Dutch researchers have undertaken a series of FMDV transmission studies that have involved sheep;
- A wide range of samples had been collected for virus isolation and serology;
- Some samples had been tested by qRT-PCR using manual extractions

Quantification of foot and mouth disease virus excretion and transmission within groups of lambs with and without vaccination

K. Orsel [a,*], A. Dekker [b], A. Bouma [a], J.A. Stegeman [a], M.C.M. de Jong [c]

[a] *Faculty of Veterinary Medicine, Department of Farm Animal Health, Utrecht University, The Netherlands*
[b] *Central Institute for Animal Disease Control Lelystad (CIDC-Lelystad), Wageningen UR, The Netherlands*
[c] *Animal Sciences Group, Wageningen University and Research Centre Wageningen, The Netherlands*

Received 30 May 2006; received in revised form 13 October 2006; accepted 23 November 2006
Available online 6 December 2006

Bravo de Rueda *et al. Veterinary Research* 2014, **45**:58
http://www.veterinaryresearch.org/content/45/1/58

VETERINARY RESEARCH

RESEARCH Open Access

Estimation of the transmission of foot-and-mouth disease virus from infected sheep to cattle

Carla Bravo de Rueda[1,2], Mart CM de Jong[2], Phaedra L Eblé[1] and Aldo Dekker[1*]

FMDV surveillance questions

- Dutch studies had shown FMDV could be isolated from blood and oral swabs;
- Virus could be isolated from oral swabs for longer than detection in blood;
- Could oral swabs be used for FMDV surveillance in sheep by using qRT-PCR in a high throughput format?
- Is there potential to pool oral swab samples for qRT-PCR testing?

FMDV research strategy

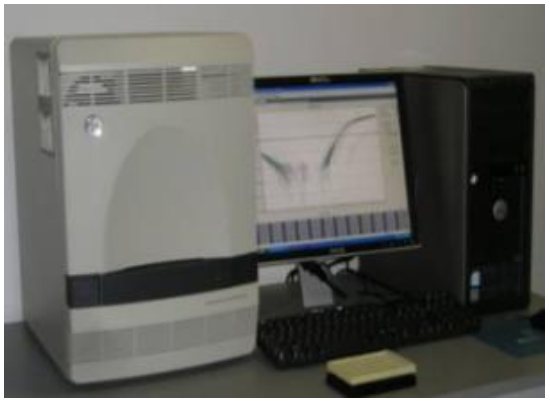
- CVI Lelystad, The Netherlands retained archival samples from all published studies;
- All samples were offered for testing but facilities at Lelystad not suitable for high through-put testing;
- Collaborating laboratory identified – FLI, Insel Reims, Germany;
- Consultation re suitable level of pooling - Will vary depending on stage of infection;
- Pooling questions – all samples on the same day? A cross sectional mix? What should be pooled – original samples? NA extracts?

FMDV project planning logistics & challenges

- Agreement on test methods (NA extraction, qRT-PCR and reagents)
- Use of internal control
- “Pre-project” testing – comparison of all reagents
- Shipment of reagents to Germany
- Sample transfer - >4000 samples across FMDV free country;
- Selection of samples for testing
- Agreement on pooling options

FMDV test methods

- Overall aim was to simulate methods in routine use at EMAI
- Sample handling – sample into MagMax lysis buffer
- NA extraction protocol – using MagMax-96 RNA kit on KF96 platform
- Assay & reagents – AgPath RT mastermix and OIE 3D assay;
- Selection of internal control – available to all labs
- Pooling – practical, rapid and takes stage of infection into account



The FLI Method Sample Inactivation

- 200 µl AL buffer into a deep well transfer plate.
- Heat sealed with adhesive foil.
- Pre- heated at + 80ºC 20 mins.
- 100 µl of sample.
- 120 µl of PBS/Protease.
- Heat sealed with foil again.
- + 80ºC 2 x 10 mins (mixed by inversion).
- Transfer plate out of BSL3 Ag (3% VENNO® VET solution) to BSL2.

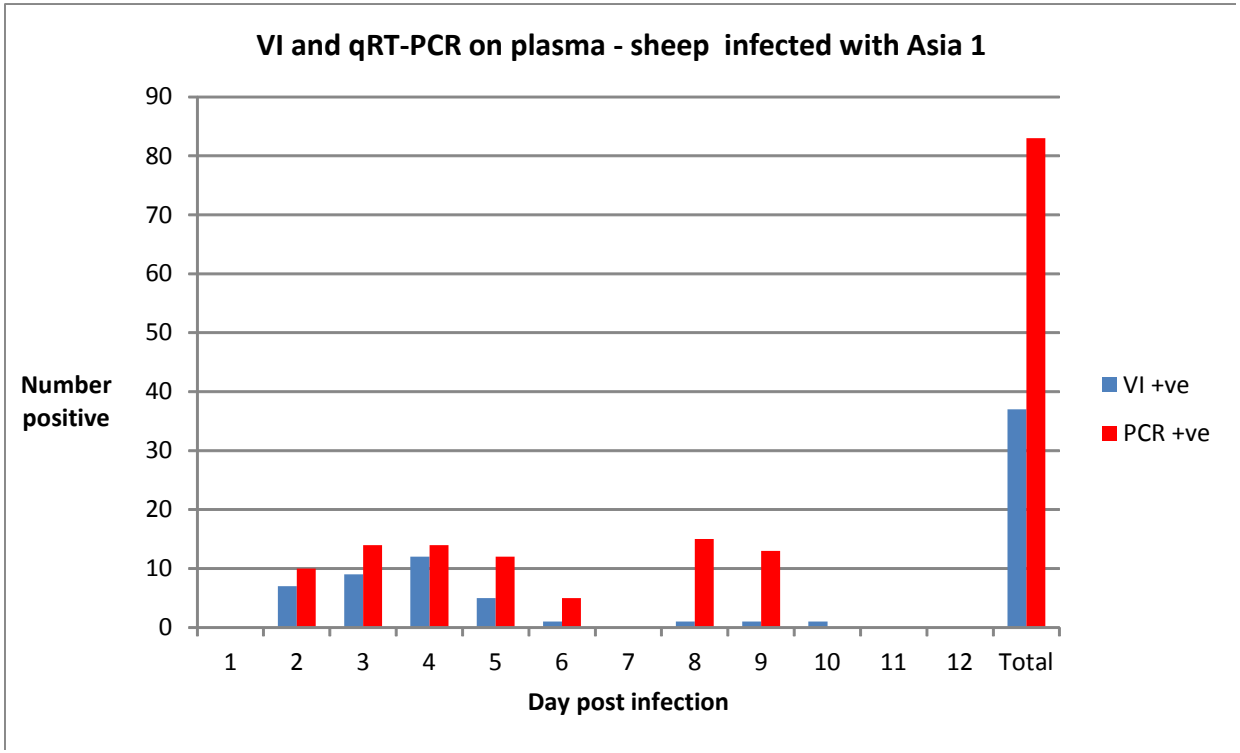
Extraction Volumes and Parameters.

	EMAI MagMAX-96	FLI Qiagen + MagMAX-96
Sample volume	50 µl	**100 µl**
Lysis buffer	130 µl	**200 µl – AL Buffer**
Heat	No	**80 ° C 2 x 10 mins**
Protease	No	**25 µl + 100 µl PBS**
Beads	20 µl	**20 µl**
Lysis/sample/bead mix	5 minutes	**4 minutes**
Ethanol wash	150 µl x 2 – 2 mins	**150 µl x 2 – 75 sec**
Isopropanol wash	150 µl x 2 – 2 mins	**150 µl x 2 – 75 sec**
Elution buffer	50 µl	**80 µl**
Mixing speed	Slow	**Fast**
Air dry	1 minute	**5 minutes**
Elution	4 minutes	**1 minute 40 seconds**

FLI Work Routine

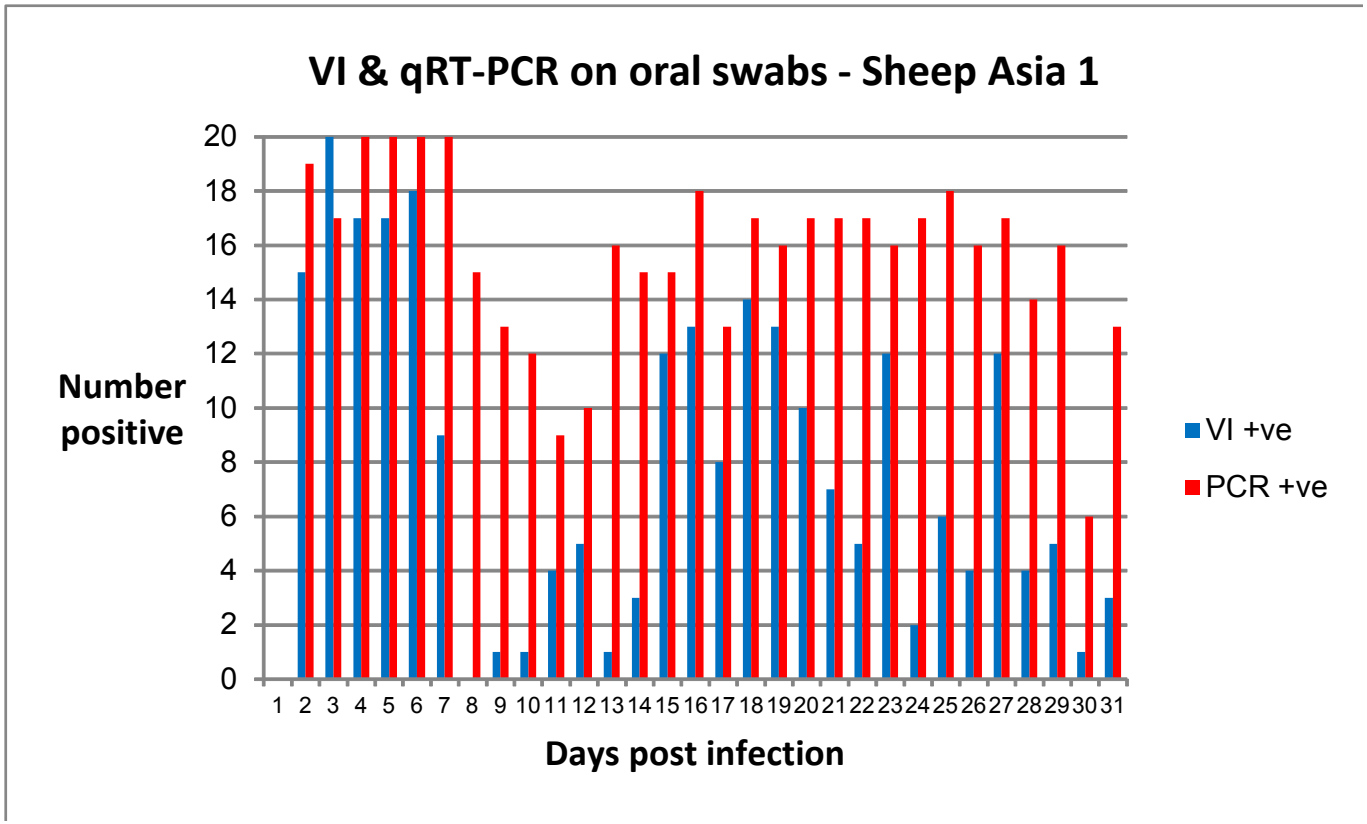
- Inactivations completed → BSL2 → extractions → PCR.
- 3 days – 3 people = 1442 samples tested.
- Results loaded into LIMS, scrutinized daily,

VI & qRT-PCR – plasma (Asia 1)



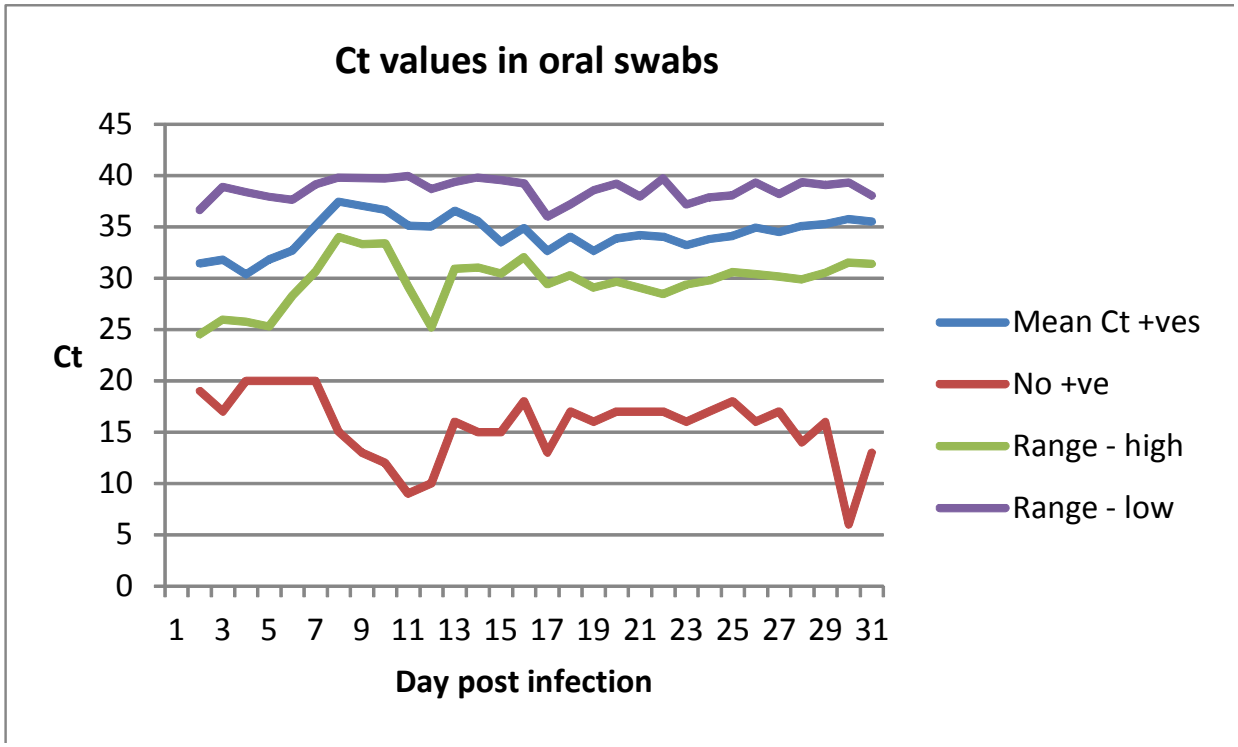
Because virus could be detected in plasma for a limited period (based on virus isolation results), qRT-PCR was only attempted on one sample set

qRT-PCR testing of oral swabs

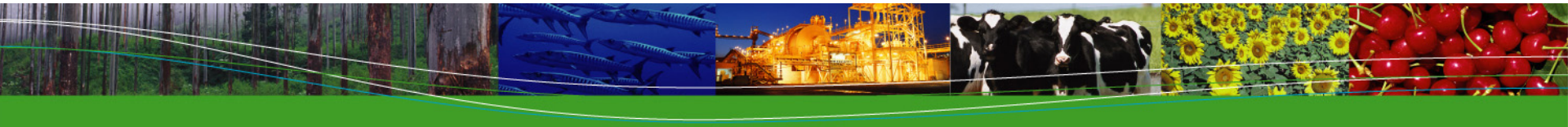


qRT-PCR detected FMDV RNA in oral swabs from a high proportion of sheep throughout the 31 day study period

qRT-PCR testing of oral swabs



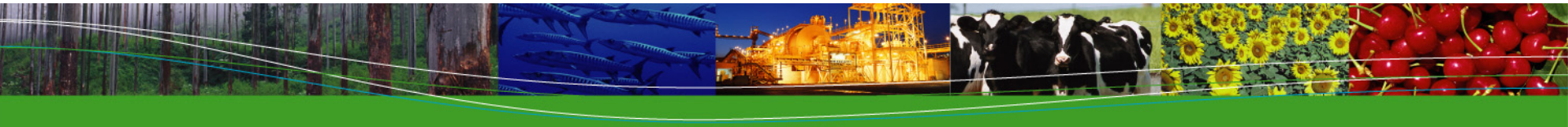
After day 10 little change in Ct values detected in the FMDV qRT-PCR over the 31 day study period



qRT-PCR – pooled oral swabs

- Pooling simulated by diluting individual positive samples in pooled RNA extract of oral swabs from Day 0
- Samples diluted for first day infection detected
- Pooling limited to 10 samples for practical reasons (little advantage from larger pools)
- All positive samples detected in pool of 10
- Ct values from pool of 10 samples not proportional to predicted results for highly efficient assay (1/10 result frequently <3.3 Ct higher than undiluted sample
- No opportunity to investigate aberrant results

	Day 2 post infection			
Sheep	Undiluted	1/3	1/5	1/10
1	24.49	25.77	26.56	27.17
2	30.59	31.78	32.43	33.15
3	35.44	37.23	38.42	38.01
4	28.04	30.38	30.35	31.29
5	34.66	35.66	35.73	36.01
6	26.14	27.67	28.23	29.04
7	30.54	31.97	32.52	33.54
8	26.13	27.20	27.97	28.66
9	40.27	Neg	Neg	Neg
10	36.48	37.81	37.60	40.01
11	34.42	35.37	35.76	36.62
12	32.49	34.00	34.27	34.47
13	34.03	34.86	35.54	37.00
14	34.84	35.34	36.52	37.46
15	33.57	33.08	34.86	35.99
16	28.91	30.33	30.07	31.23
17	32.14	32.03	34.08	34.92
18	31.03	Neg	32.46	Neg
19	36.62	38.08	38.14	39.03
20	26.24	27.26	28.04	28.70



qRT-PCR – pooled oral swabs

- Samples diluted to 1/5 for samples near end of sampling period;
- Day 28 chosen to give maximum number of positive samples (little day to day change)
- Pooling limited to 5 samples - larger pools less likely to detect infection??
- All samples detected in pool of 5 samples
- Samples giving very high Ct values remarkably consistent after dilution – is maximum quantity of RNA being detected??
- XIPC remarkably stable
- No opportunity to investigate further

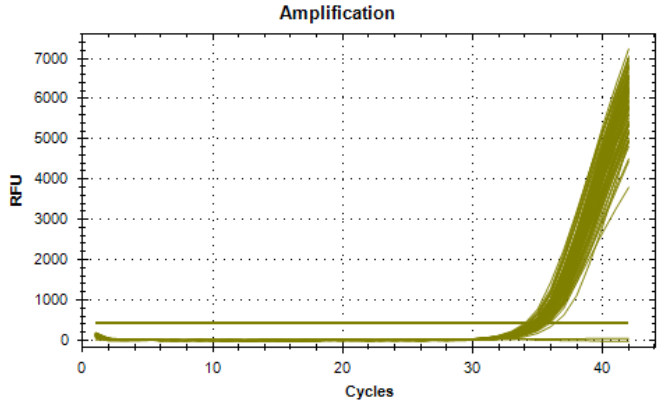
	Day 28		
Sheep	Undiluted	1/3	1/5
1	36.00	37.34	37.72
2	Neg	NT	NT
3	37.32	38.64	38.66
4	36.16	37.71	37.39
5	38.99	39.07	39.8
6	40.01	NT	NT
7	37.01	38.02	39.24
8	34.86	36.58	37.27
9	30.84	32.35	32.89
10	35.24	36.87	37.1
11	39.06	39.37	38.6
12	34.79	33.1	36.73
13	30.50	37.26	33.64
14	36.27	NT	NT
15	41.01	Neg	Neg
16	34.03	36.02	35.95
17	30.62	32.17	32.85
18	36.00	36.79	37.12
19	Neg	NT	NT
20	36.61	39.38	38.43

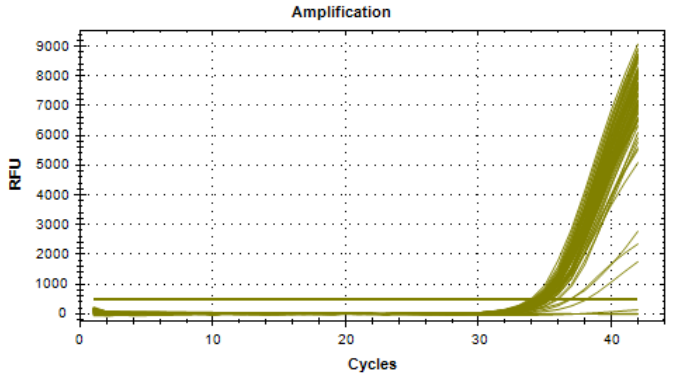
qRT-PCR – Internal control

- Due to local logistics, the exogenous internal positive control was not used in the standard format.
- The XIPC was still added to samples prior to extraction
- Very high degree of reproducibility for the XIPC with individual samples:

  99.2% of samples within range (12/1442 failed)

  (no opportunity to investigate)
- For pooled samples, all samples within range:
  Range 34.03-36.35; Mean 35.05, SD 0.43





Impact of buffer combinations

- Pre-project evaluation of the Official protocol indicated a lack of compatibility between buffers from different kits (cf RLT buffer).
- Results suggest suboptimal extraction and presence of inhibitors (eg blood)
- Possible solutions: standard sample volumes, wash times & elution conditions,

	AL Buffer-FLI extraction		AL Buffer into MMX		Modified + 50uL elution		Modified + 80uL elution	
	A (PBS)	B (blood)	A (PBS)	B (blood)	A (PBS)	B (blood)	A (PBS)	B (blood)
PEV dilution								
10^-1	35.44	37.58	no Cq	38.86	34.07	39.01	34.91	39.03
10^-2	40.94	no Cq	no Cq	no Cq	36.03	no Cq	38.03	35.84
10^-3	37.26	no Cq	no Cq	no Cq	33.45	no Cq	35.94	no Cq
10^-4	36.33	39.00	no Cq	no Cq	33.92	no Cq	34.76	38.93
10^-5	no Cq	no Cq	no Cq	no Cq	35.32	no Cq	34.13	no Cq
10^-6	36.10	no Cq	no Cq	no Cq	35.73	no Cq	37.53	38.95
10^-7	37.73	no Cq	no Cq	no Cq	39.20	no Cq	35.00	38.13
10^-8	39.13	no Cq	no Cq	no Cq	37.47	no Cq	37.23	38.17

FMDV project challenges & next steps

- Results on pooled samples suggest suboptimal amplification of RNA
- Need to evaluate effects of heating and compare alternative time/temperatures
- Compare standard EMAI extraction protocol with modified FLI-EMAI protocol
- Need to re-evaluate FMDV inactivation with standard lysis buffers with/without heating at lower temperatures

The FMD Research Team

- CVI Lelystad, The Netherlands

Aldo Dekker, Phaedre Eble

WAGENINGEN
UNIVERSITY & RESEARCH

- Institute of Diagnostic Virology,

Friederich Loeffler Institute, Insel Reims, Germany

Holger Scholten, Anja Schultz, Kerstin Wernike, Martin Beer, Bernd Haas

- Virology Laboratory, EMAI

Rodney Davis & Peter Kirkland.

Funding: NSW DPI, McGarvie Smith Animal Health Trust, MLA Donor Company

FRIEDRICH-LOEFFLER-INSTITUT
FLI
Bundesforschungsinstitut für Tiergesundheit
Federal Research Institute for Animal Health


Thank you for your attention