Multiplex Real-Time RT-PCR for Detection of FMDV, Rift Valley Fever Virus and Bovine Viral Diarrhea Virus in Bulk Tank Milk

Michael Eschbaumer and Kerstin Wernike

Institute of Diagnostic Virology,
Friedrich-Loeffler-Institut
Greifswald-Insel Riems, Germany

eufmd
European Commission for the control of FMD

Rev. sci. tech. Off. int. Epiz., **16** (1), 117-124

Risks of spreading foot and mouth disease through milk and dairy products

A.I. Donaldson

Institute for Animal Health, Pirbright Laboratory, Pirbright, W

Summary

A review of epidemics of foot and mouth disease (FMD) has highlighted th
important role which raw (untreated) milk can play in the spread of the disease i
a country which is normally free of FMD and whose cattle are not routinel

Modeled detection time for surveillance for foot-and-mouth disease virus in bulk tank milk

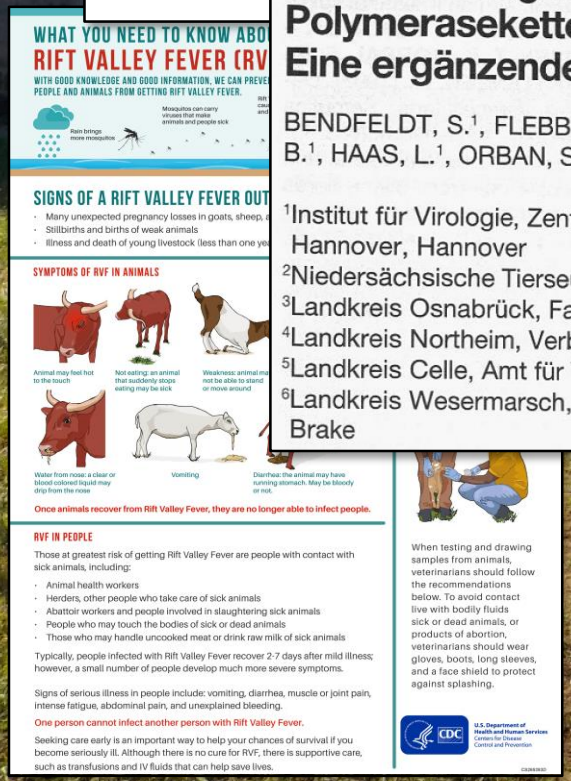
Mark C. Thurmond,

Conclusions and Clinical Relevance—PCR screening of bulk milk for FMDV would likely detect FMDV in dairy herds several days sooner than might be expected for owner reporting of clinical signs and worthy of consideration for regional, al FMD surveillance. (*Am J Vet Res* 024)

Untersuchung von Sammelmilchproben mittels Polymerasekettenreaktion: Eine ergänzende Methode für die BVD-Überwachung

BENDFELDT, S.[1], FLEBBE, U.[2], FRITZEMEIER, J.[3], GREISER-WILKE, I.[1], GRUMMER, B.[1], HAAS, L.[1], ORBAN, S.[4], PETERS, E.[5], TIMM, D.[6], MOENNIG, V.[1]*

[1]Institut für Virologie, Zentrum für Infektionsmedizin der Tierärztlichen Hochschule Hannover, Hannover
[2]Niedersächsische Tierseuchenkasse, Hannover
[3]Landkreis Osnabrück, Fachdienst Tiere und Lebensmittel, Osnabrück
[4]Landkreis Northeim, Verbraucherschutz- und Veterinäramt, Northeim
[5]Landkreis Celle, Amt für Veterinärangelegenheiten und Verbraucherschutz, Celle
[6]Landkreis Wesermarsch, Amt für Veterinärwesen und Lebensmittelüberwachung, Brake



RVF IN PEOPLE

Those at greatest risk of getting Rift Valley Fever are people with contact with sick animals, including:

- Animal health workers
- Herders, other people who take care of sick animals
- Abattoir workers and people involved in slaughtering sick animals
- People who may touch the bodies of sick or dead animals
- Those who may handle uncooked meat or drink raw milk of sick animals

Journal of Virological Methods 217 (2015) 28–35

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

RVFV Drosten mix 2 TexasRed (Drosten et al., 2002, J Clin Microbiol 40[7]:2323-30)

40 µl	RVS-F (100 pmol/µl)	*5'-AAA GGA ACA ATG GAC TCT GGT CA-3'*
40 µl	RVAs-R (100 pmol/µl)	*5'-CAC TTC TTA CTA CCA TGT CCT CCA AT-3'*
8 µl	RVP-TEX (100 pmol/µl)	*5'-TexasRed-AAA GCT TTG ATA TCT CTC AGT GCC CCA A-BHQ2-3'*
112 µl	0.1X TE	
200 µl		

β-actin mix 2 (To

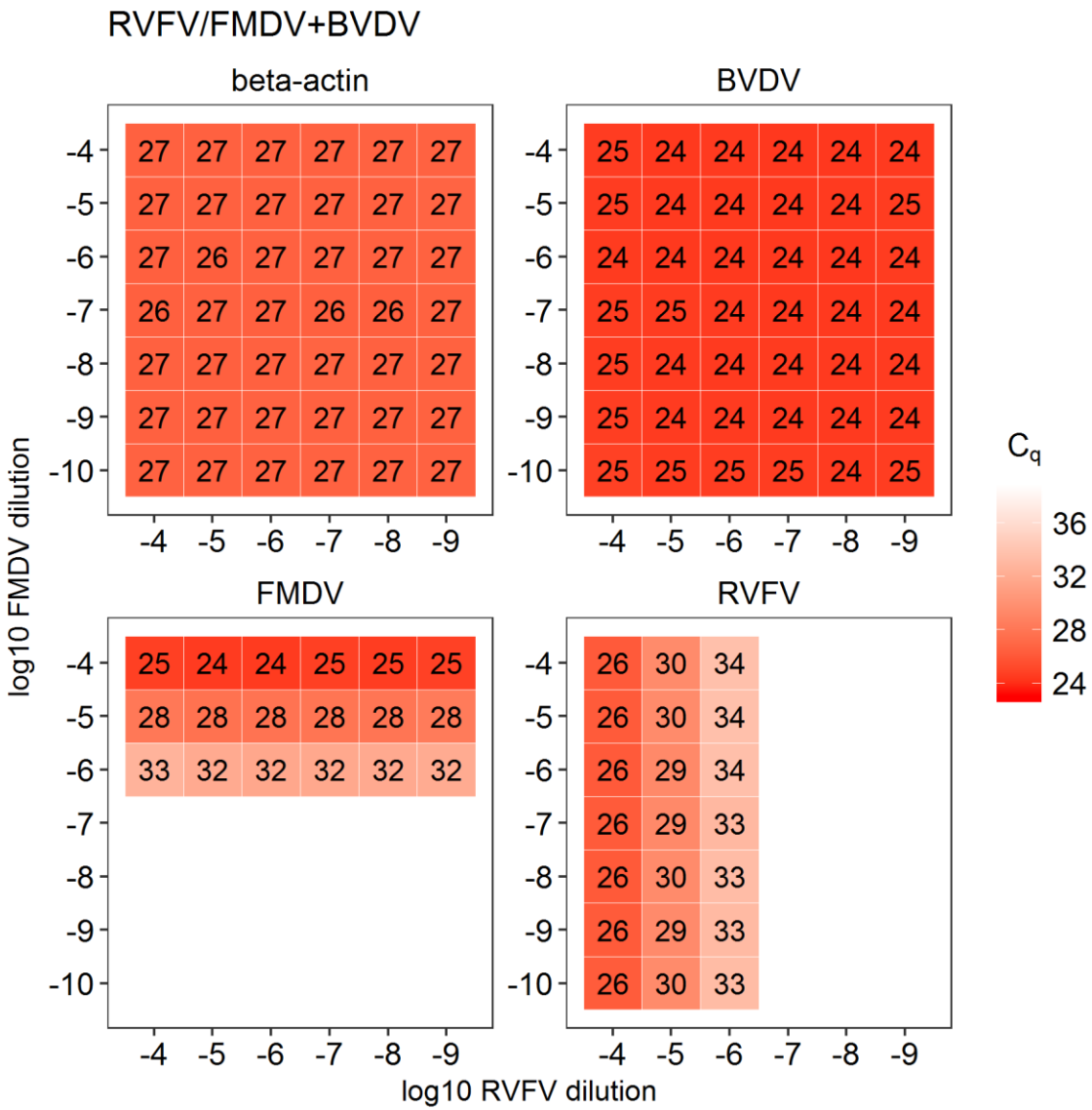
5 µl	ACT-10
5 µl	ACT-11
2.5 µl	ACT-10
187.5 µl	0.1X TE
200 µl	

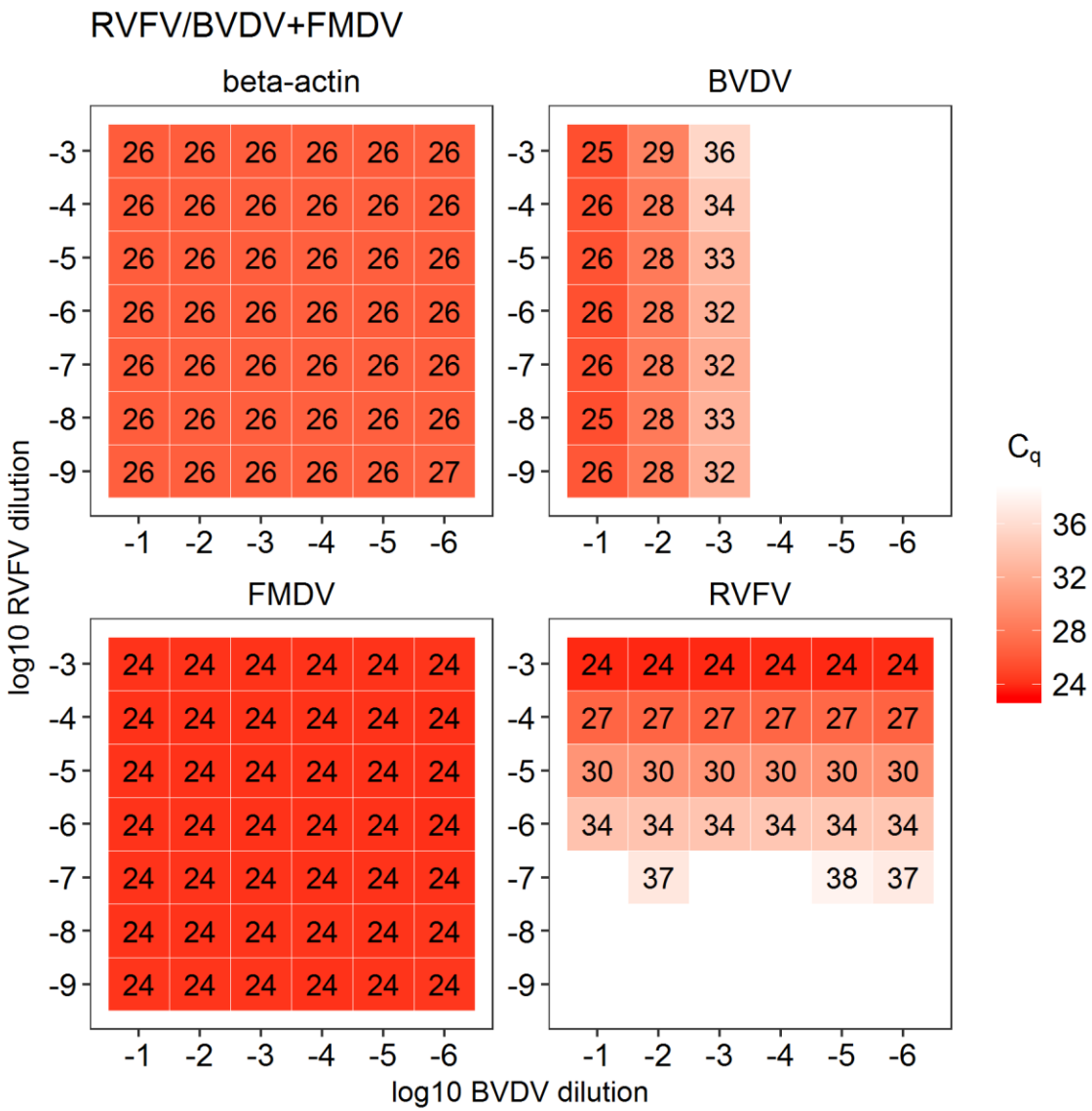
BVDV PP-1.1 mix 2 Cy5 (Hoffmann et al., 2006, J Virol Methods 136[1-2]:200-9)

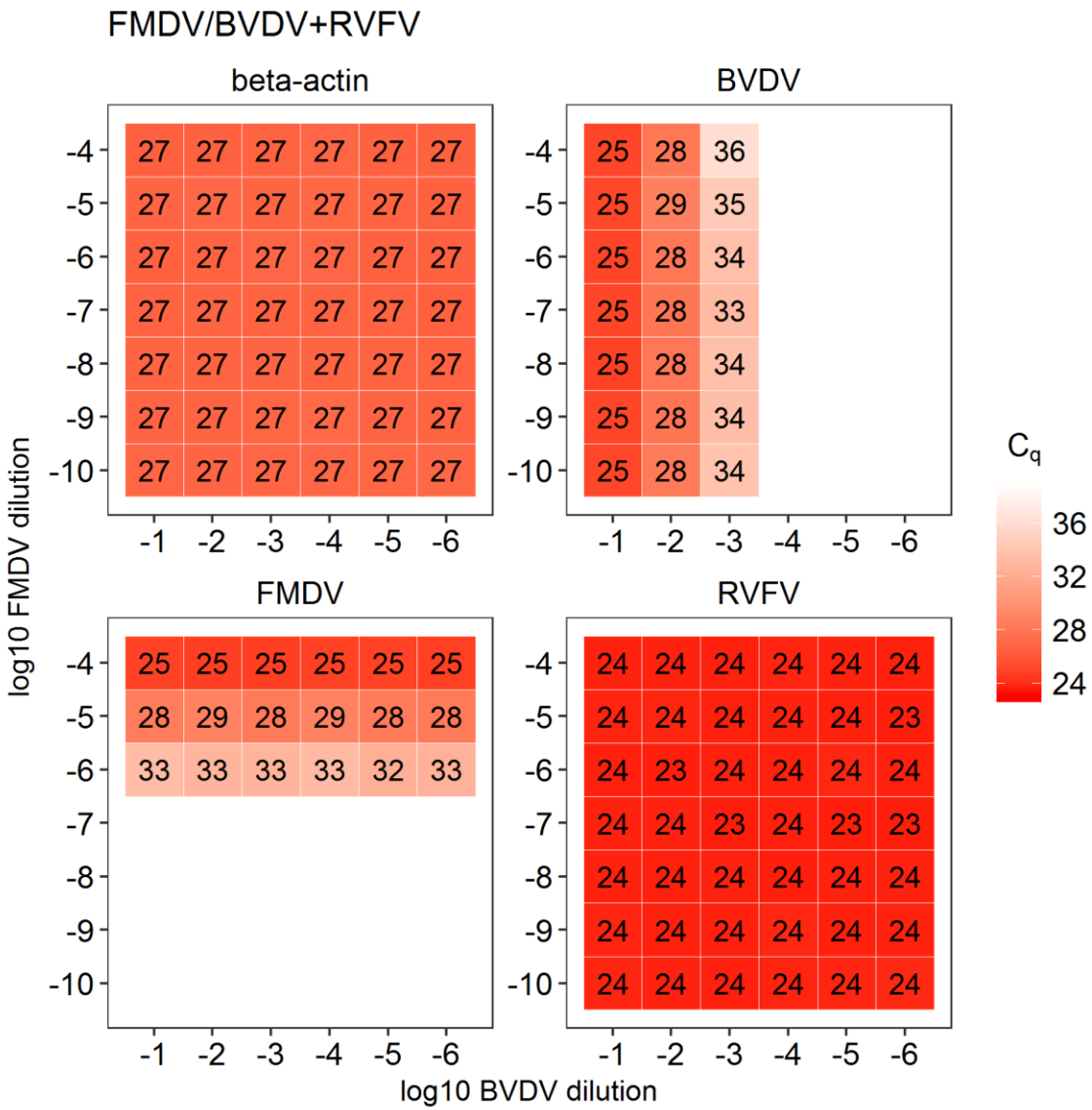
80 µl	BVD190-F (100 pmol/µl)	*5'-GRA GTC GTC ART GGT TCG AC-3'*
80 µl	V326 (100 pmol/µl)	*5'-TCA ACT CCA TGT GCC ATG TAC-3'*
12 µl	TQ-Pesti-Cy5 (100 pmol/µl)	*5'-Cy5-TGC YAY GTG GAC GAG GGC ATG C-BHQ3-3'*
28 µl	0.1X TE (pH 8.0)	
200 µl		

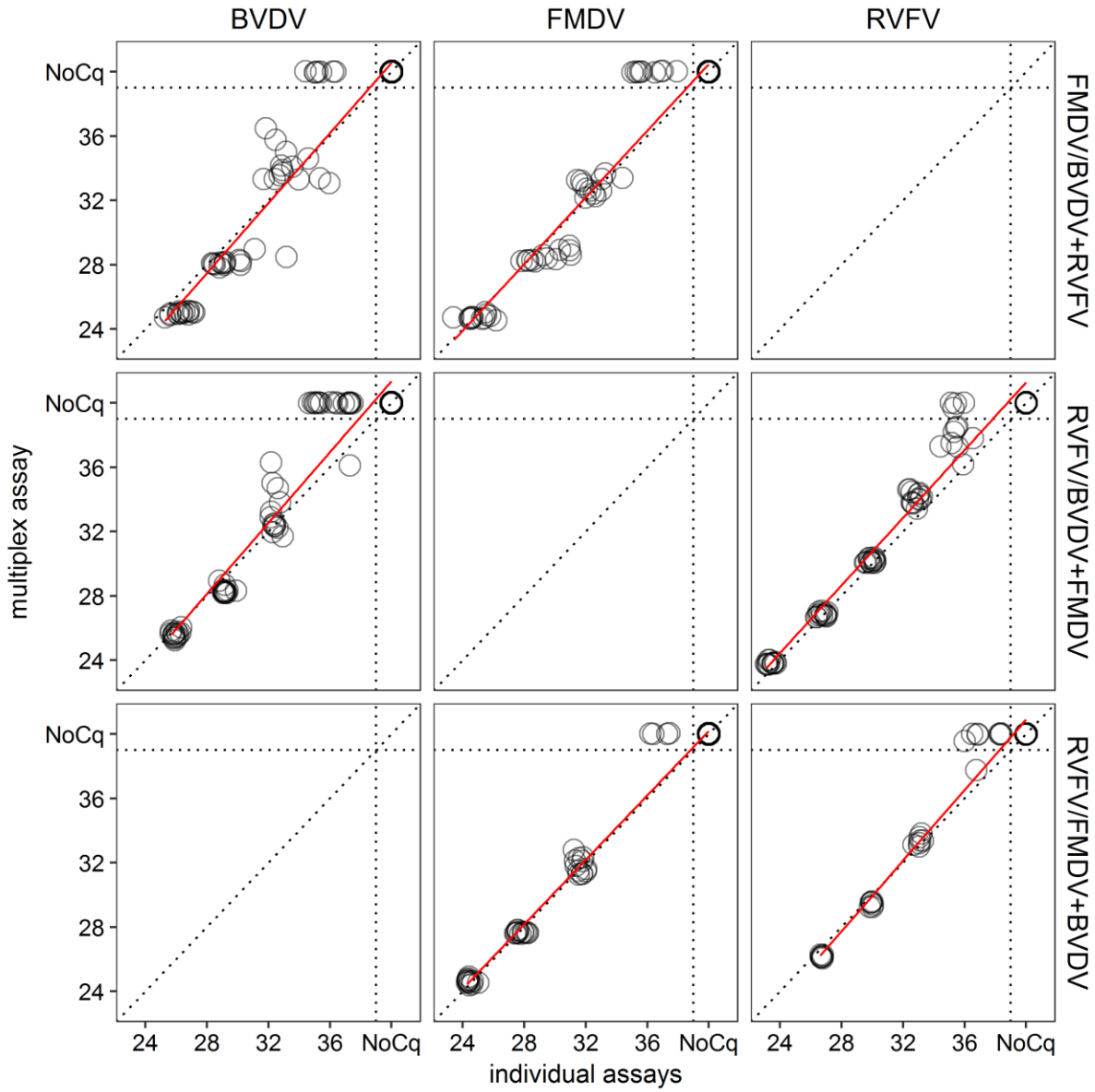
FMDV IRES 4G mix 3 FAM (Wernike et al., 2013, J Clin Microbiol 51[3]:938-44)

60 µl	FMD-IRES-4.1F (100 pmol/µl)	*5'-TAA CAW GGA CCC RCS GGG CC-3'*
60 µl	FMD-IRES-4R (100 pmol/µl)	*5'-TGA AGG GCA TCC TTA GCC TG-3'*
12 µl	FMD-IRES-4.1TEX (100 pmol/µl)	*5'-FAM-CAT GTG TGC AAY CCC AGC ACR G-BHQ1-3'*
68 µl	0.1X TE (pH 8.0)	
200 µl		


RVFV/FMDV+BVDV
beta-actin
BVDV
FMDV
RVFV
log10 FMDV dilution
log10 RVFV dilution
Cq
36
32
28
24


RVFV/BVDV+FMDV
beta-actin
BVDV
FMDV
RVFV
log10 RVFV dilution
log10 BVDV dilution
Cq
36
32
28
24


FMDV/BVDV+RVFV
beta-actin
BVDV
FMDV
RVFV
log10 FMDV dilution
log10 BVDV dilution
-4
-5
-6
-7
-8
-9
-10
-1
-2
-3
-4
-5
-6
27 27 27 27 27 27
27 27 27 27 27 27
27 27 27 27 27 27
27 27 27 27 27 27
27 27 27 27 27 27
27 27 27 27 27 27
27 27 27 27 27 27
25 28 36
25 29 35
25 28 34
25 28 33
25 28 34
25 28 34
25 28 34
25 25 25 25 25 25
28 29 28 29 28 28
33 33 33 33 32 33
24 24 24 24 24 24
24 24 24 24 24 23
24 23 24 24 24 24
24 24 23 24 23 23
24 24 24 24 24 24
24 24 24 24 24 24
24 24 24 24 24 24
Cq
36
32
28
24


BVDV
FMDV
RVFV
FMDV/BVDV+RVFV
RVFV/BVDV+FMDV
RVFV/FMDV+BVDV
multiplex assay
individual assays
NoCq
36
32
28
24

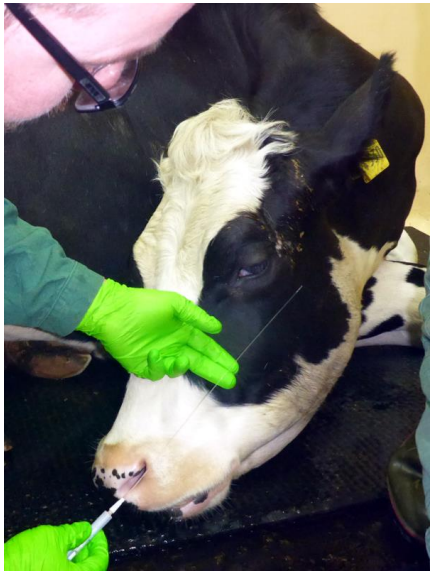
O1 Manisa	3D-1	IRES4G	A22 Iraq	3D-1	IRES4G	Asia1 Shamir	3D-1	IRES4G	C1 Noville	3D-1	IRES4G
10^-1	20,2	21,1	10^-1	18,6	19,5	10^-1	20,1	18,9	10^-1	18,5	18,7
10^-2	24,0	24,3	10^-2	22,1	23,1	10^-2	23,4	22,2	10^-2	22,1	22,3
10^-3	27,0	27,7	10^-3	25,5	26,3	10^-3	26,3	25,3	10^-3	25,5	25,7
10^-4	31,4	32,8	10^-4	28,8	30,3	10^-4	30,1	29,2	10^-4	28,7	29,7
10^-5	33,1	41,0	10^-5	31,9	35,5	10^-5	34,1	34,8	10^-5	32,1	34,9
10^-6	NoCq	NoCq	10^-6	34,3	NoCq	10^-6	38,1	NoCq	10^-6	37,5	NoCq

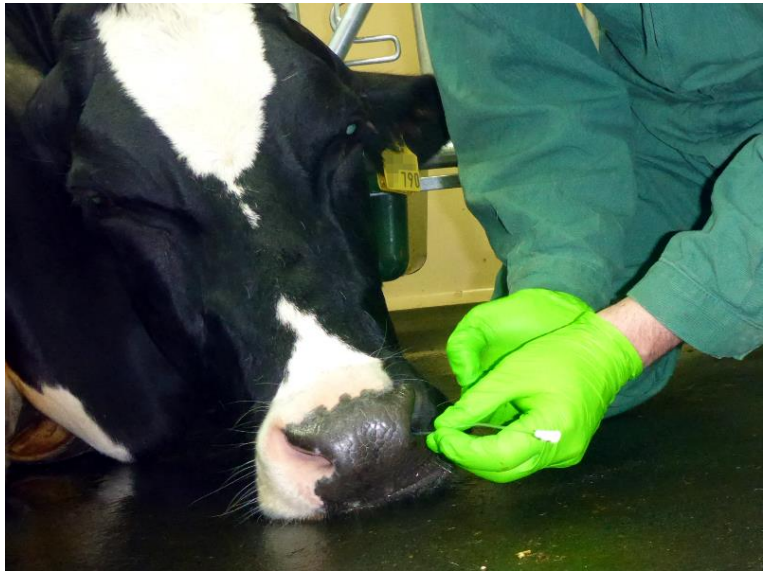
SAT1	3D-1	IRES4G	SAT2	3D-1	IRES4G	SAT3	3D-1	IRES4G
10^-1	19,2	19,3	10^-1	20,0	19,1	10^-1	18,8	18,7
10^-2	23,0	22,8	10^-2	23,5	22,4	10^-2	22,5	22,2
10^-3	26,2	26,1	10^-3	26,6	25,6	10^-3	25,4	25,4
10^-4	29,7	30,4	10^-4	31,3	30,7	10^-4	29,0	29,3
10^-5	32,1	35,2	10^-5	32,6	34,4	10^-5	32,2	33,4
10^-6	35,1	NoCq	10^-6	38,1	NoCq	10^-6	36,5	NoCq

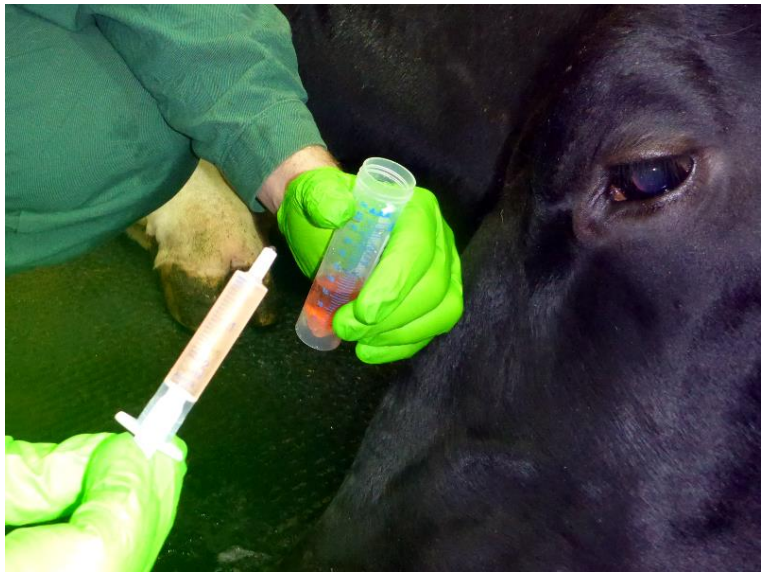
species	strain	subtype	C_q value
BVDV-1	NADL	1a	27.3
BVDV-1	Paplitz	1b	27.6
BVDV-1	NCP-2508-FCS	1c	29.2
BVDV-1	PI809	1d	28.0
BVDV-1	NC3807-1251/1	1e	28.2
BVDV-1	Egbert	1f	29.1
BVDV-1	BO806-17	1g	28.4
BVDV-1	BO807-3	1h	28.5
BVDV-1	Böhni	1k	30.0
BVDV-1	NC3807-8757	1x	28.1
BVDV-2	8644	2a	28.6
BVDV-2	Bure	2a	28.3
BVDV-2	Walter	2b	28.4
BVDV-2	PO1600	2c	28.0

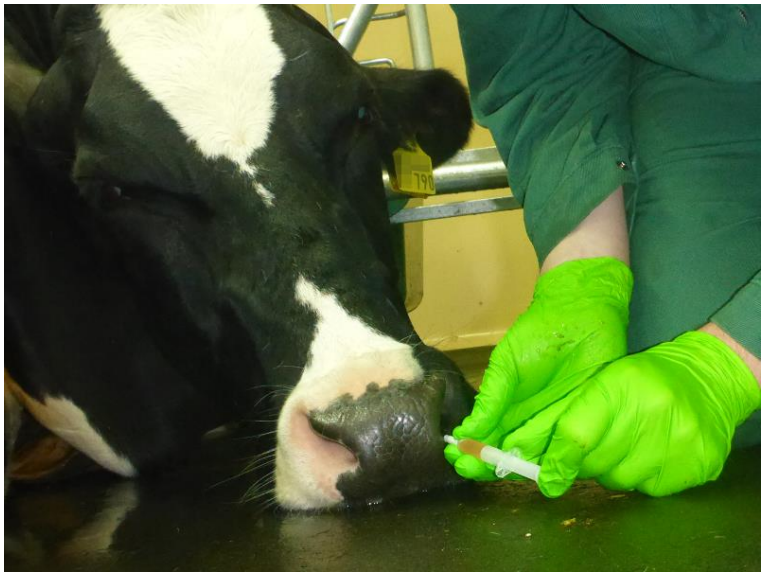
3 dairy cows,
2½, 2½ and 9 years
late lactation

infection by
intranasopharyngeal
application of FMDV
A/IRN/22/2015 in
deep sedation





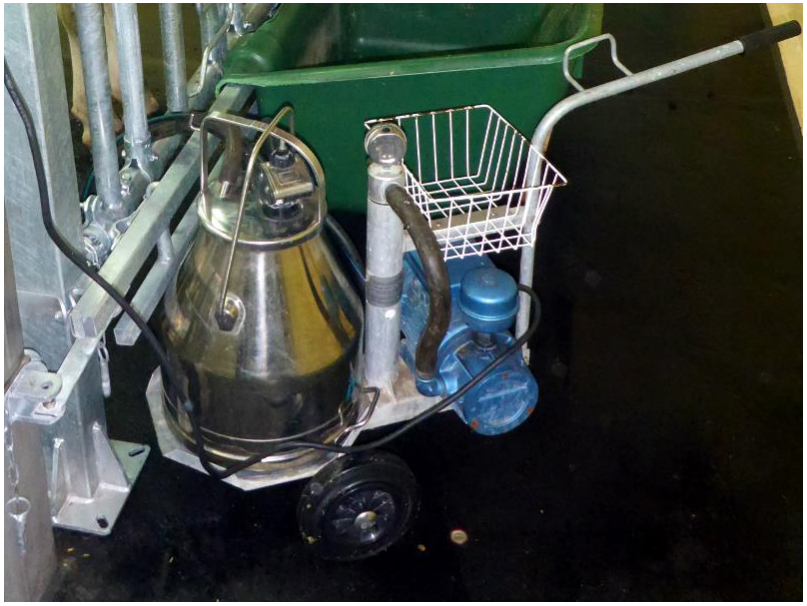


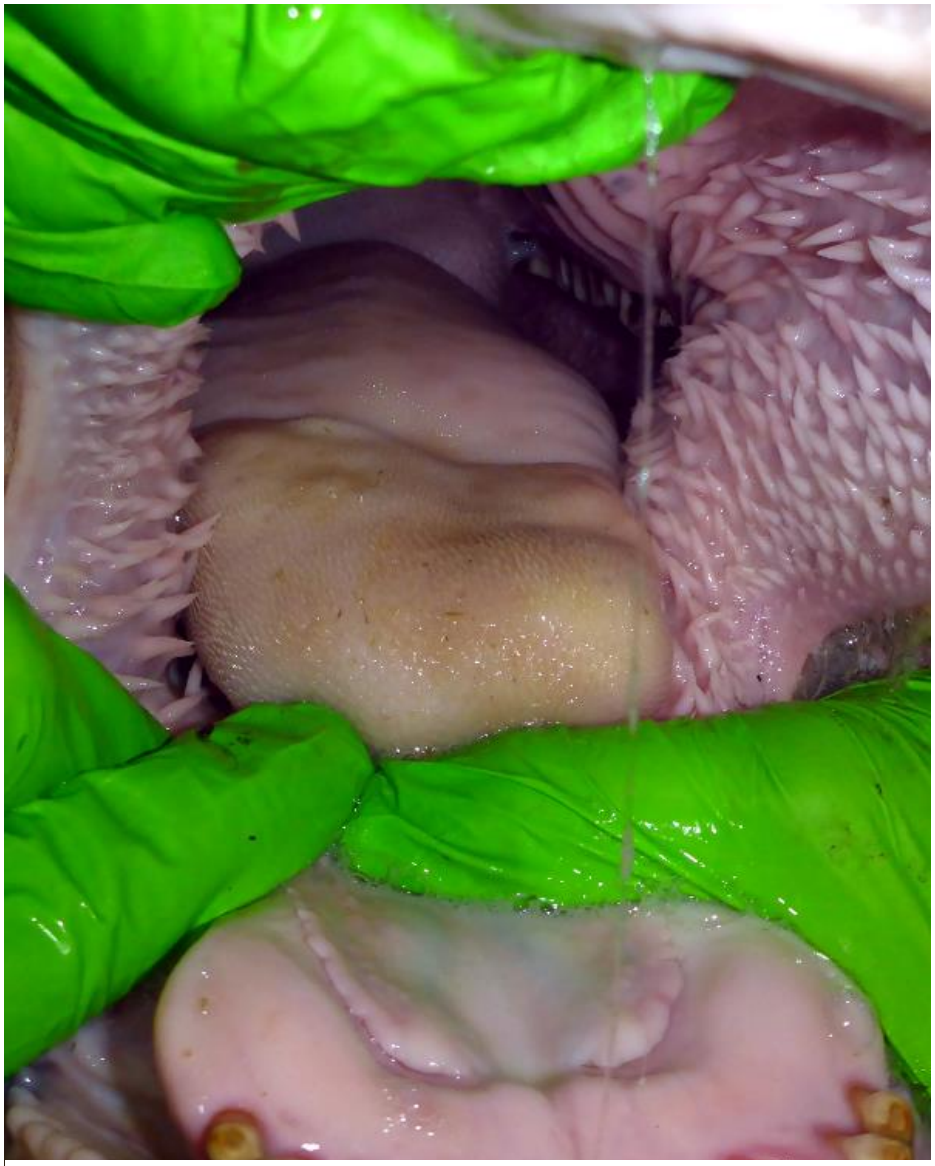


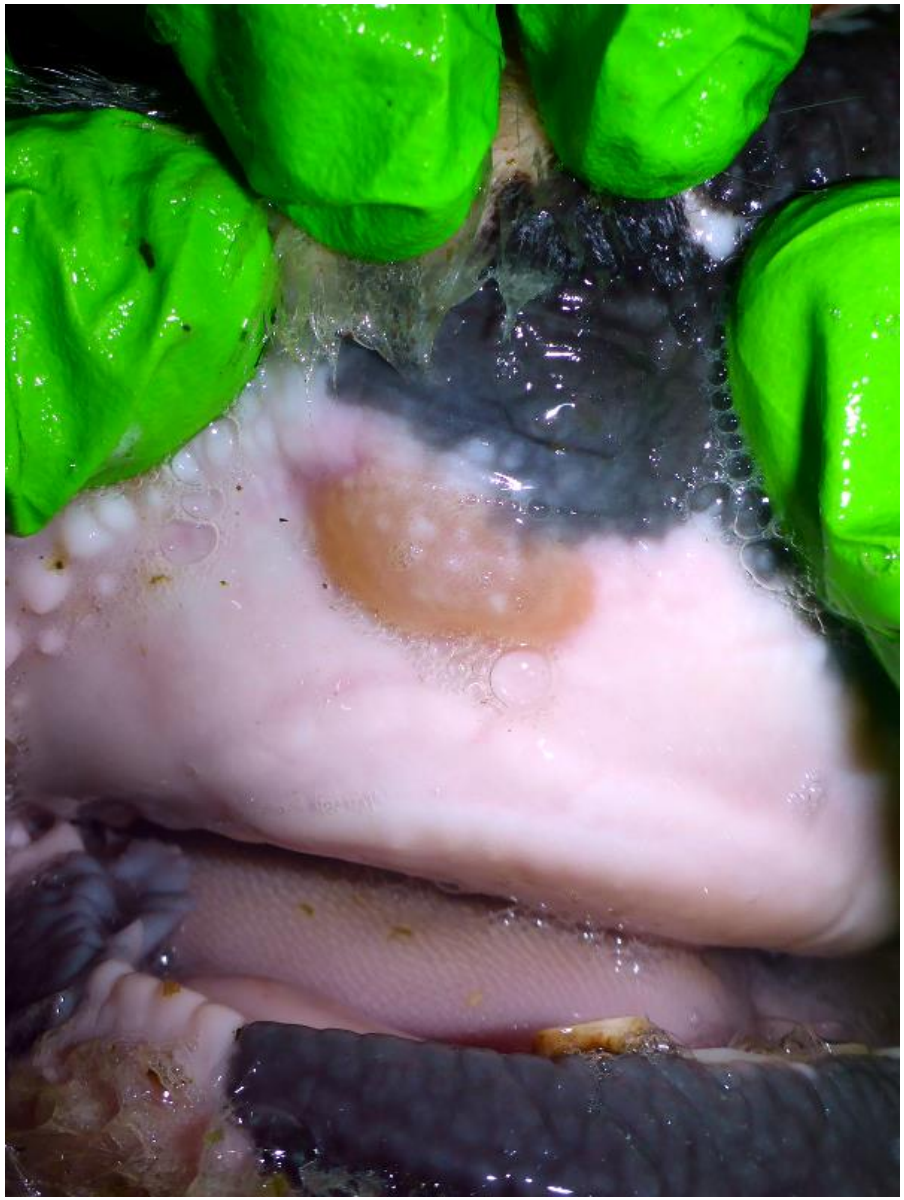
small portable milking machine, thoroughly cleaned and disinfected between animals

1 daily milking in the morning

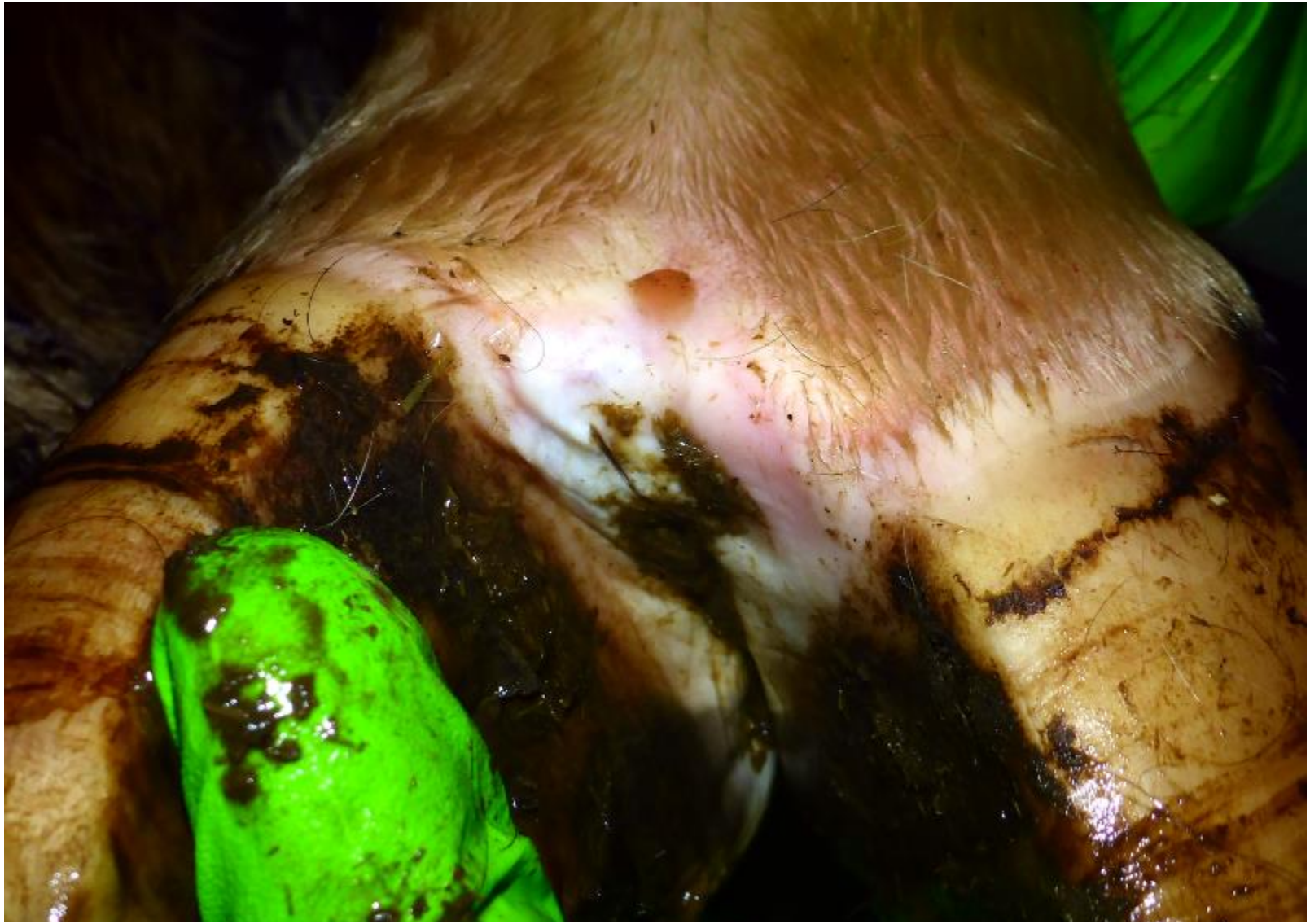
collection of milk, blood, saliva and nasal fluid for 12 days



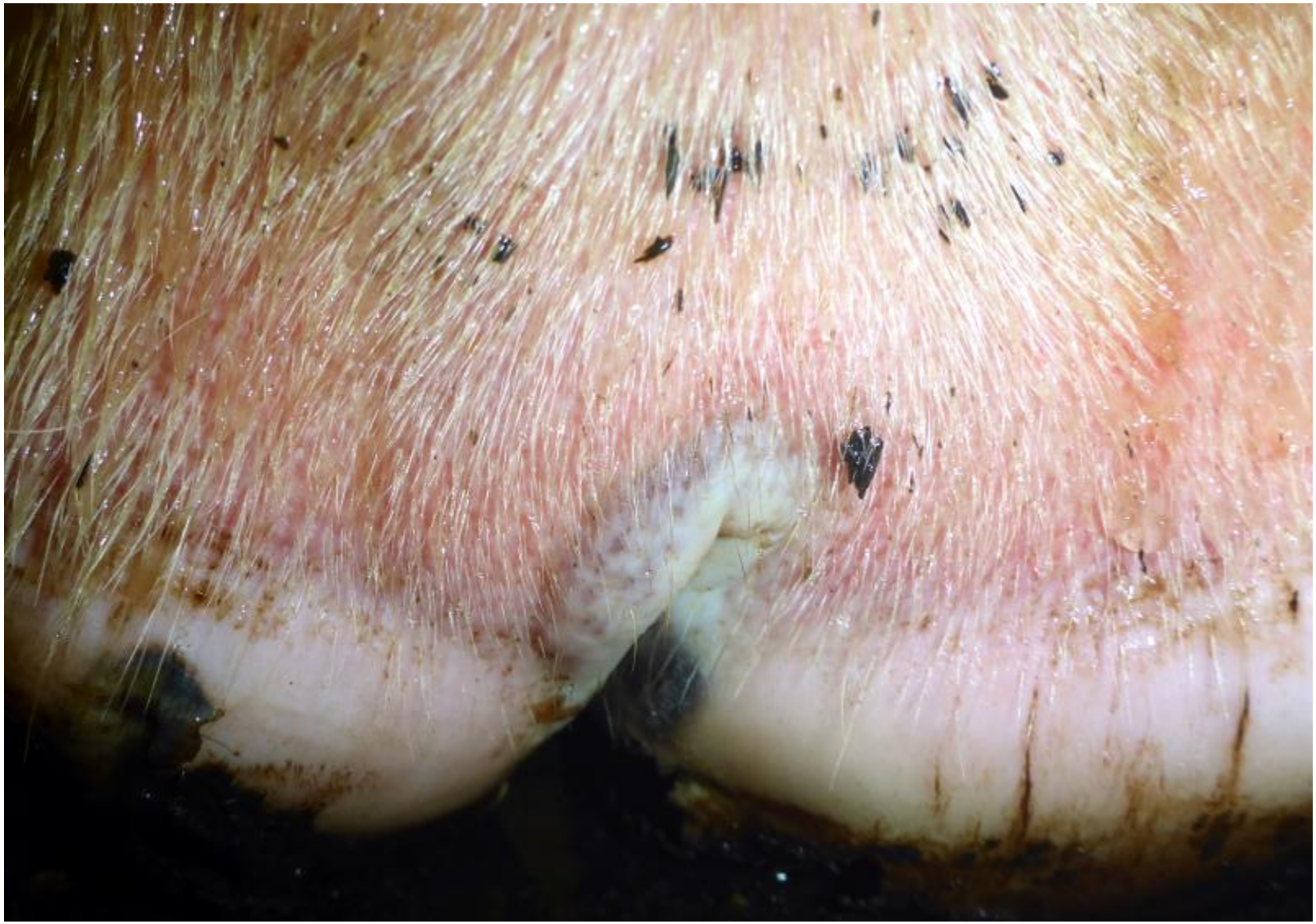




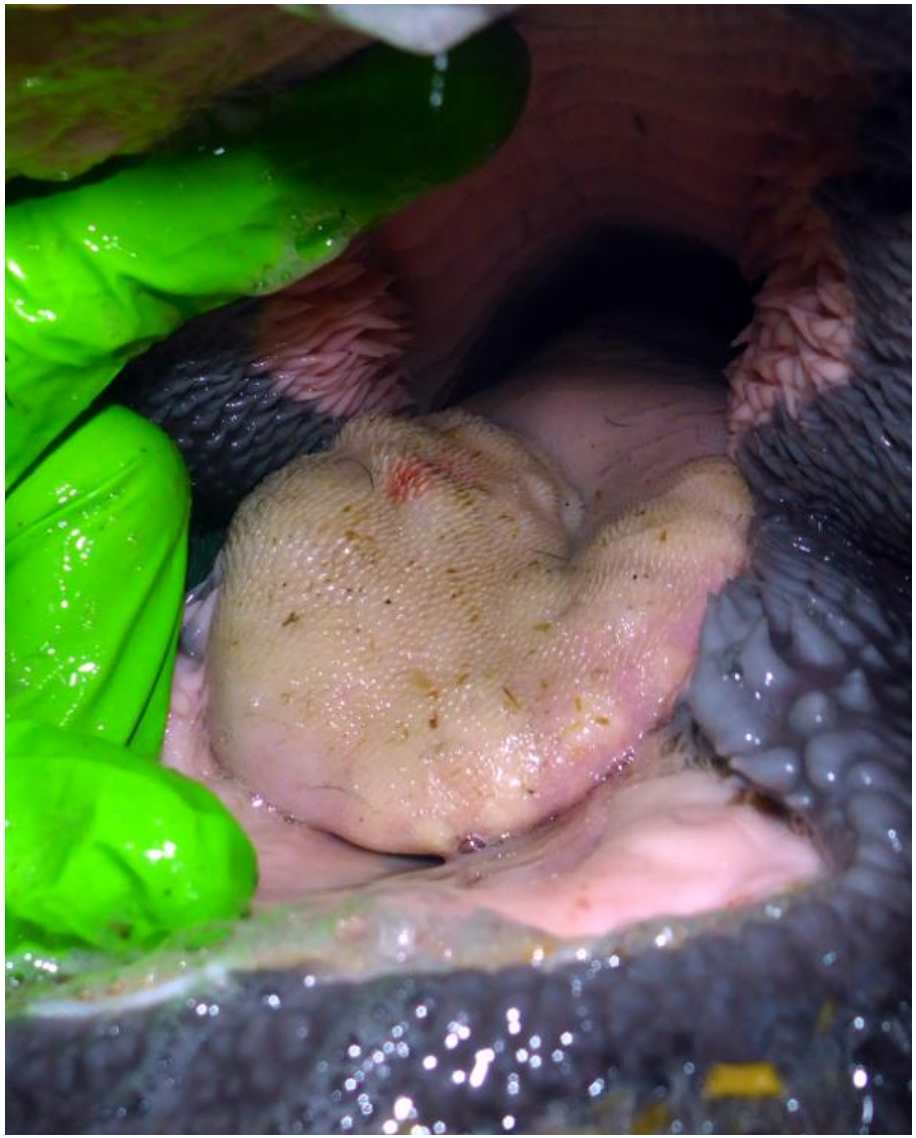
cow 530, 3 dpi, mouth



cow 542, 3 dpi, interdigital space

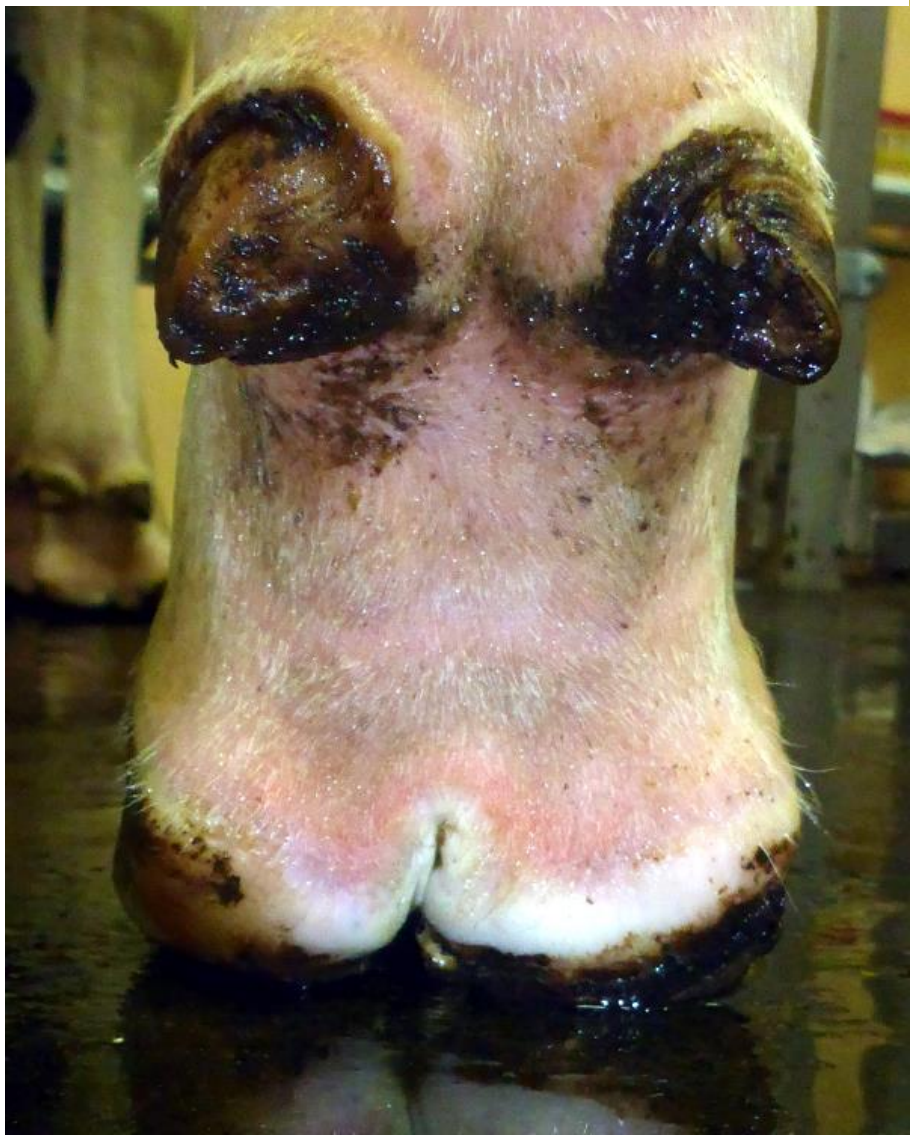


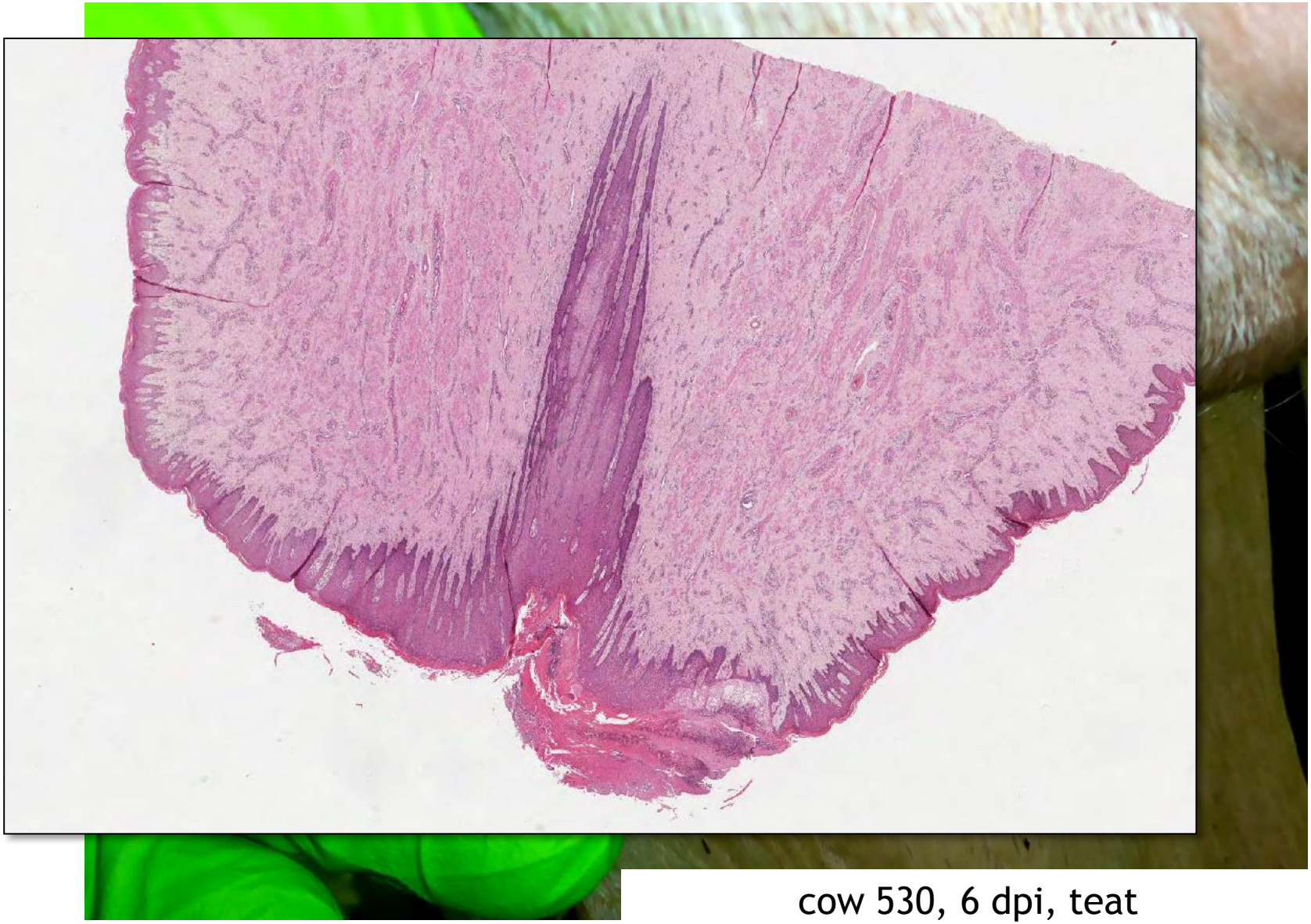
cow 542, 3 dpi, interdigital space



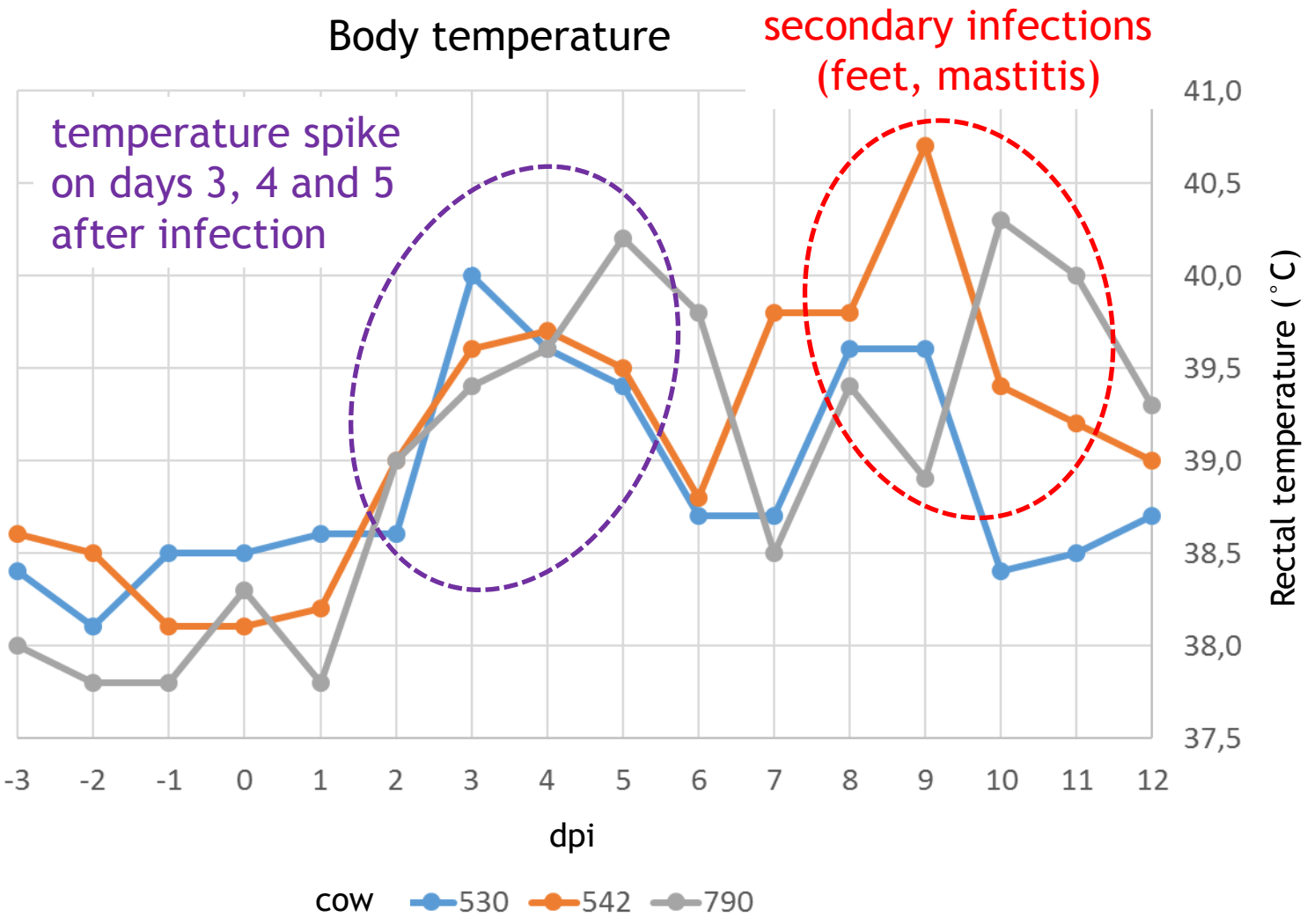
cow 790, 3 dpi, tongue

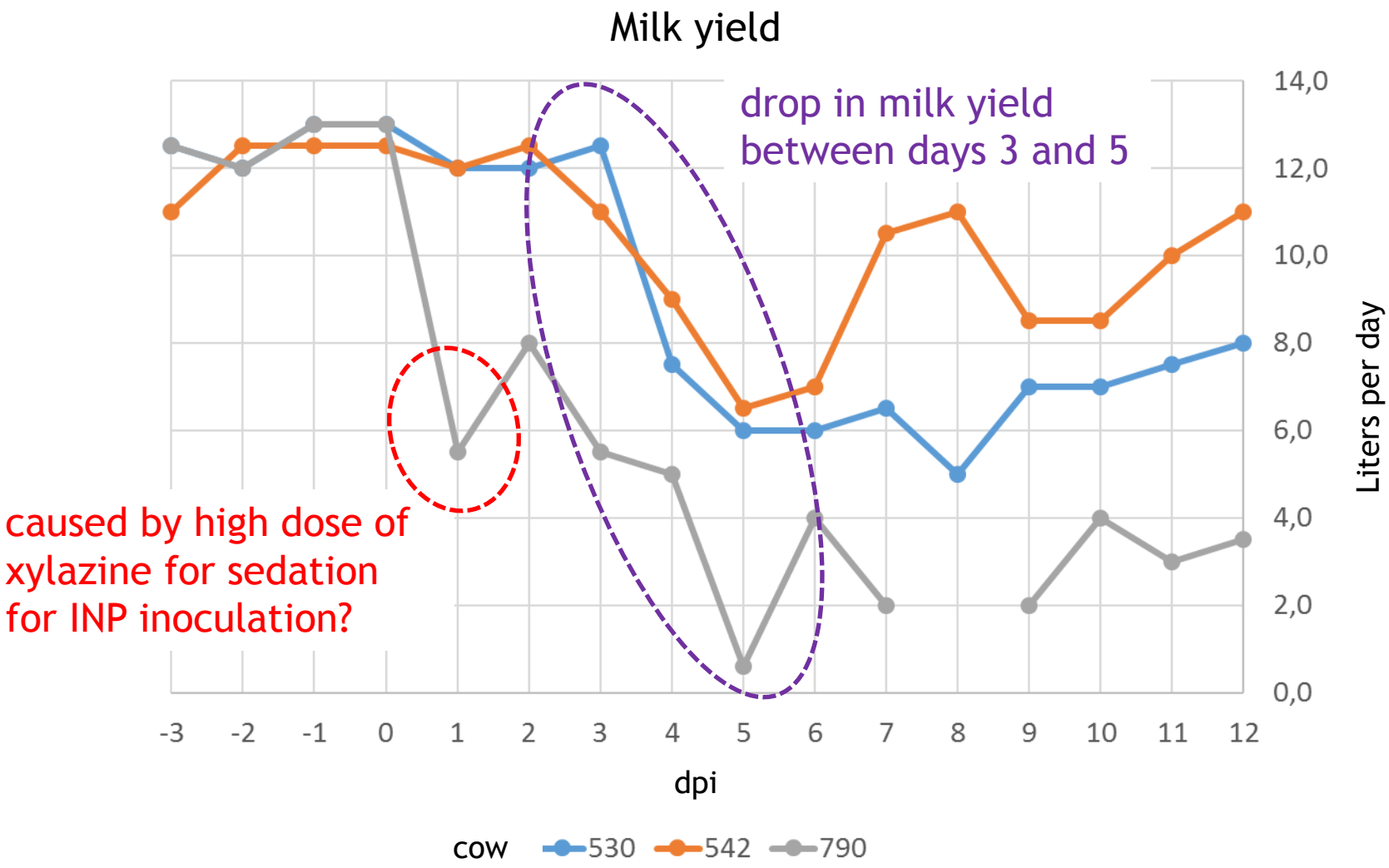
cow 530, 4 dpi



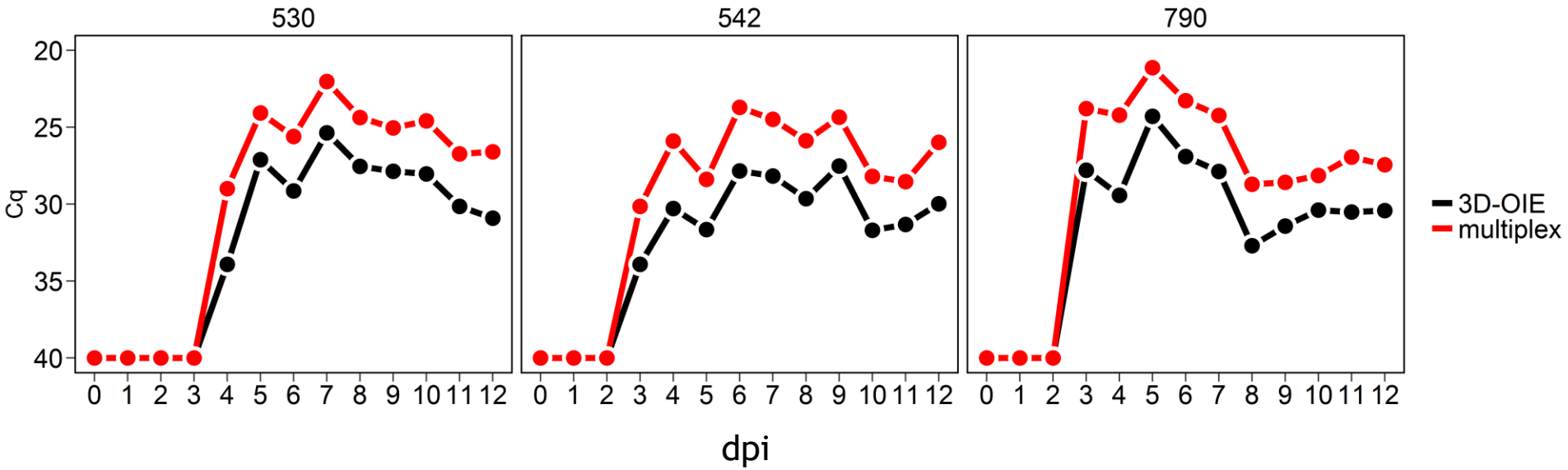


cow 530, 6 dpi, teat

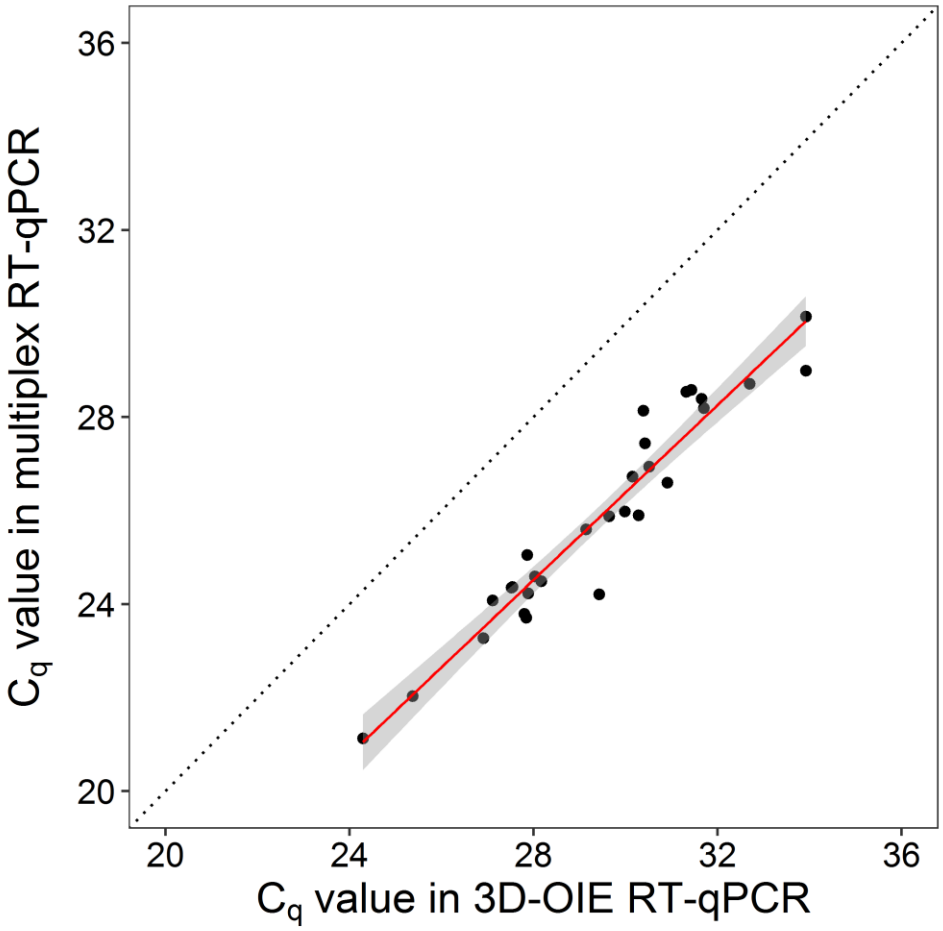

Body temperature
secondary infections
(feet, mastitis)
temperature spike
on days 3, 4 and 5
after infection
41,0
40,5
40,0
39,5
39,0
38,5
38,0
37,5
Rectal temperature (°C)
-3
-2
-1
0
1
2
3
4
5
6
7
8
9
10
11
12
dpi
cow
530
542
790

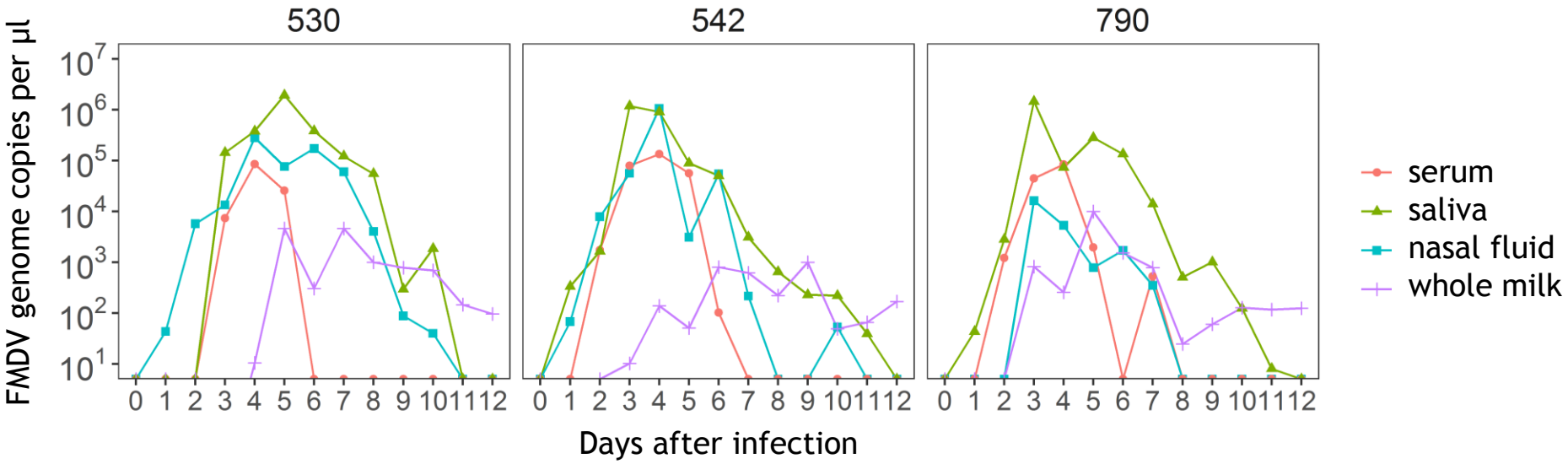

Milk yield
drop in milk yield
between days 3 and 5
caused by high dose of
xylazine for sedation
for INP inoculation?
14,0
12,0
10,0
8,0
6,0
4,0
2,0
0,0
Liters per day
-3
-2
-1
0
1
2
3
4
5
6
7
8
9
10
11
12
dpi
cow
530
542
790

FMDV PCR from whole milk

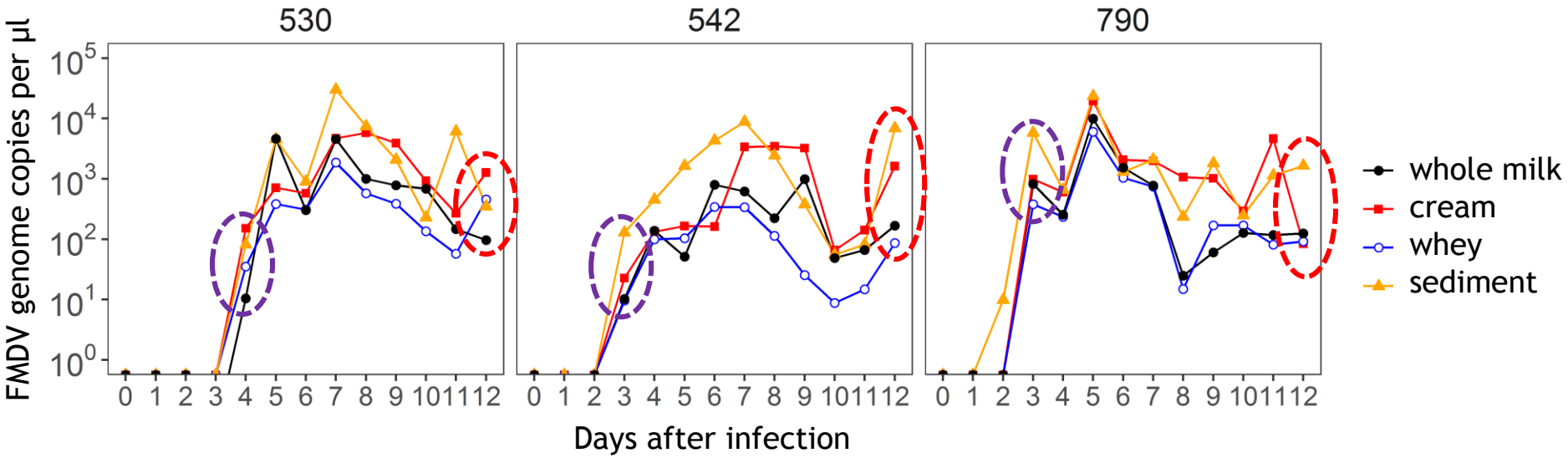


FMDV PCR from whole milk




530
542
790
FMDV genome copies per µl
10^7
10^6
10^5
10^4
10^3
10^2
10^1
0 1 2 3 4 5 6 7 8 9 10 11 12
Days after infection
serum
saliva
nasal fluid
whole milk

virus detectable in milk until the end of the experiment (12 dpi)



first detection of FMDV genome in milk on day 3 or 4 after infection, coinciding with first clinical signs

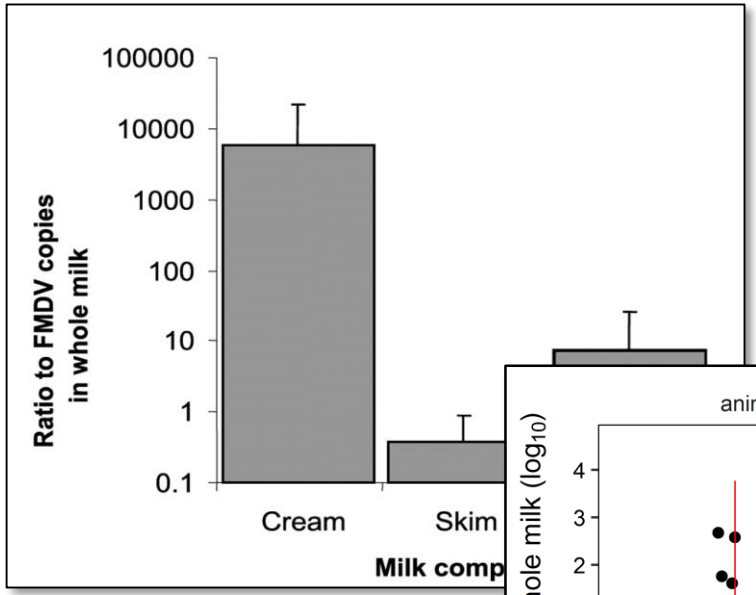
roughly equal distribution of FMDV genome across all milk fractions

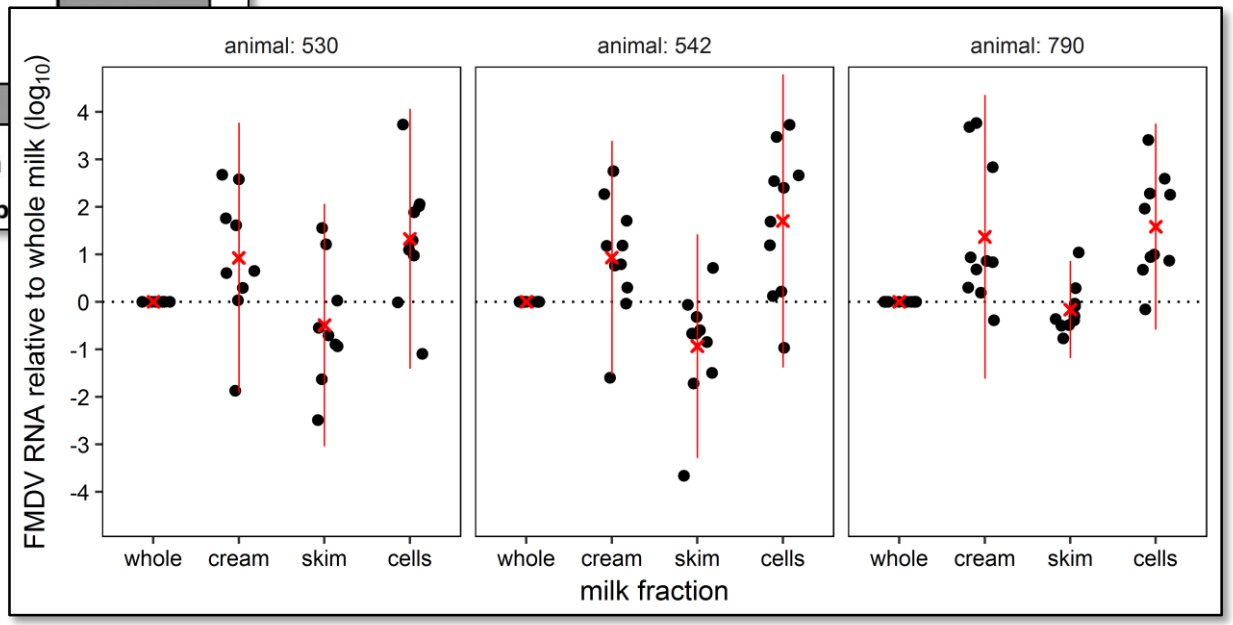
Vet. Res. 37 (2006) 121–132
© INRA, EDP Sciences, 2005
DOI: 10.1051/vetres:2005040

Original article

Utility of automated real-time RT-PCR for the detection of foot-and-mouth disease virus excreted in milk

Scott M. REID[a]*, Satya PARIDA[a], Donald P. KING[a], Geoffrey H. HUTCHINGS[a], Andrew E. SHAW[a], Nigel P. FERRIS[a], Zhidong ZHANG[a], J. Eric HILLERTON[b], David J. PATON[a]





235 milk samples from Kenya…

		multiplex RT-qPCR				3D-OIE	
FLI ID	TPI ID	FMDV Cq	BVDV Cq	RVFV Cq	β-actin Cq	FMDV Cq	β-actin Cq
1	AA 001	NoCq	NoCq	NoCq	27.5	NoCq	30.9
2	BB 001	NoCq	NoCq	NoCq	27.3	NoCq	30.3
3	CC 001	NoCq	NoCq	NoCq	27.0	NoCq	30.3
4	DD 001	NoCq	NoCq	NoCq	26.9	NoCq	30.2
5	EE 001	NoCq	NoCq	NoCq	26.3	NoCq	29.6
6	AA 002	NoCq	NoCq	NoCq	27.7	NoCq	31.1
7	BB 002	NoCq	NoCq	NoCq	27.2	NoCq	30.9
8	CC 002	NoCq	NoCq	NoCq	27.1	NoCq	30.2
9	DD 002	NoCq	NoCq	NoCq	26.7	NoCq	30.3
10	EE 002	NoCq	NoCq	NoCq	25.8	NoCq	29.3
11	FF 002	NoCq	NoCq	NoCq	27.1	NoCq	30.4
13	BB 003	NoCq	NoCq	NoCq	26.7	NoCq	30.1
15	DD 003	NoCq	NoCq	NoCq	26.4	NoCq	30.0
16	EE 003	NoCq	NoCq	NoCq	27.2	NoCq	30.7
17	FF 003	NoCq	NoCq	NoCq	27.4	NoCq	30.4
19	BB 004	NoCq	NoCq	NoCq	26.8	NoCq	29.9
20	CC 004	NoCq	NoCq	NoCq	27.0	NoCq	29.8
21	DD 004	NoCq	NoCq	NoCq	27.3	NoCq	30.7
22	EE 004	NoCq	NoCq	NoCq	26.8	NoCq	30.2
23	FF 004	NoCq	NoCq	NoCq	27.6	NoCq	31.0
25	BB 005	NoCq	NoCq	NoCq	27.4	NoCq	30.6
26	CC 005	NoCq	NoCq	NoCq	27.1	NoCq	30.0
27	DD 005	NoCq	NoCq	NoCq	27.3	NoCq	30.5
28	EE 005	NoCq	NoCq	NoCq	27.4	NoCq	30.9
29	FF 005	NoCq	NoCq	NoCq	27.0	NoCq	30.3
31	AA 006	NoCq	NoCq	NoCq	26.7	NoCq	30.6
32	BB 011	NoCq	NoCq	NoCq	25.6	NoCq	30.1
33	CC 011	NoCq	NoCq	NoCq	26.2	NoCq	30.8
34	DD 011	NoCq	NoCq	NoCq	26.2	NoCq	29.9
35	EE 011	NoCq	NoCq	NoCq	25.6	NoCq	29.9
36	FF 011	NoCq	NoCq	NoCq	25.8	NoCq	29.6
38	BB 012	NoCq	NoCq	NoCq	25.1	NoCq	29.5
39	CC 012	NoCq	NoCq	NoCq	25.6	NoCq	29.9
40	DD 012	NoCq	NoCq	NoCq	25.1	NoCq	29.4
41	EE 012	NoCq	NoCq	NoCq	25.8	NoCq	30.3
42	FF 012	NoCq	NoCq	NoCq	26.9	NoCq	31.0
44	BB 013	NoCq	NoCq	NoCq	25.1	NoCq	29.6
45	CC 013	NoCq	NoCq	NoCq	26.1	NoCq	30.0
46	DD 013	NoCq	NoCq	NoCq	26.0	NoCq	30.3
47	EE 013	NoCq	NoCq	NoCq	25.3	NoCq	29.2
48	FF 013	NoCq	NoCq	NoCq	25.5	NoCq	29.7
50	BB 014	NoCq	NoCq	NoCq	25.1	NoCq	29.8
51	CC 014	NoCq	NoCq	NoCq	25.7	NoCq	30.2
52	DD 014	NoCq	NoCq	NoCq	25.6	NoCq	30.0
53	EE 014	NoCq	NoCq	NoCq	26.2	NoCq	30.4
54	FF 014	NoCq	NoCq	NoCq	25.5	NoCq	30.1
56	BB 015	NoCq	NoCq	NoCq	25.7	NoCq	29.7
57	CC 015	NoCq	NoCq	NoCq	26.0	NoCq	29.8
58	DD 015	NoCq	NoCq	NoCq	26.1	NoCq	30.1
59	EE 015	NoCq	NoCq	NoCq	24.9	NoCq	29.7
60	FF 015	NoCq	NoCq	NoCq	25.7	NoCq	30.3
62	BB 016	NoCq	NoCq	NoCq	25.8	NoCq	30.3
63	CC 016	NoCq	NoCq	NoCq	25.7	NoCq	30.2
65	EE 016	NoCq	NoCq	NoCq	25.9	NoCq	30.5
66	FF 016	NoCq	NoCq	NoCq	25.6	NoCq	30.1

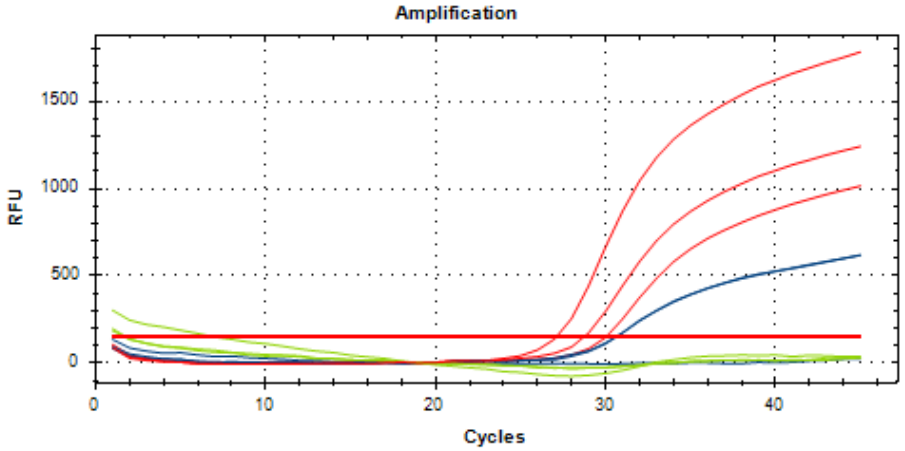
		multiplex RT-qPCR				3D-OIE	
FLI ID	TPI ID	FMDV Cq	BVDV Cq	RVFV Cq	β-actin Cq	FMDV Cq	β-actin Cq
68	BB 017	NoCq	NoCq	NoCq	26.5	NoCq	30.7
69	CC 017	NoCq	NoCq	NoCq	25.4	NoCq	29.8
70	DD 017	NoCq	NoCq	NoCq	25.7	NoCq	29.9
71	EE 017	NoCq	NoCq	NoCq	25.3	NoCq	29.1
72	FF 017	NoCq	NoCq	NoCq	25.3	NoCq	29.7
74	BB 018	NoCq	NoCq	NoCq	25.0	NoCq	29.6
75	CC 018	NoCq	NoCq	NoCq	26.1	NoCq	30.1
76	DD 018	NoCq	NoCq	NoCq	25.2	NoCq	29.9
77	EE 018	NoCq	NoCq	NoCq	24.5	NoCq	29.0
78	FF 018	NoCq	NoCq	NoCq	25.0	NoCq	29.2
79	AA 019	NoCq	NoCq	NoCq	25.6	NoCq	30.1
80	BB 019	NoCq	NoCq	NoCq	25.6	NoCq	29.7
81	CC 019	NoCq	NoCq	NoCq	24.8	NoCq	29.0
82	DD 019	NoCq	NoCq	NoCq	25.3	NoCq	29.4
83	EE 019	NoCq	NoCq	NoCq	25.4	NoCq	29.3
84	FF 019	NoCq	NoCq	NoCq	26.3	NoCq	30.2
85	AA 020	NoCq	NoCq	NoCq	28.3	NoCq	32.6
86	BB 020	NoCq	NoCq	NoCq	28.2	NoCq	32.2
87	CC 020	NoCq	NoCq	NoCq	26.2	NoCq	30.6
88	DD 020	NoCq	NoCq	NoCq	27.4	NoCq	32.0
89	EE 020	NoCq	NoCq	NoCq	28.2	NoCq	33.0
90	FF 020	NoCq	NoCq	NoCq	29.5	NoCq	34.0
91	AA 021	NoCq	NoCq	NoCq	27.1	NoCq	32.0
92	BB 021	NoCq	NoCq	NoCq	26.6	n.d.	n.d.
93	CC 021	NoCq	NoCq	NoCq	27.2	n.d.	n.d.
94	DD 021	NoCq	NoCq	NoCq	26.6	n.d.	n.d.
95	EE 021	NoCq	NoCq	NoCq	25.6	n.d.	n.d.
96	FF 021	NoCq	NoCq	NoCq	27.0	n.d.	n.d.
98	BB 022	NoCq	NoCq	NoCq	26.4	n.d.	n.d.
99	CC 022	NoCq	NoCq	NoCq	27.2	n.d.	n.d.
100	DD 022	NoCq	NoCq	NoCq	25.8	n.d.	n.d.
101	EE 022	NoCq	NoCq	NoCq	27.5	n.d.	n.d.
102	FF 022	NoCq	NoCq	NoCq	25.4	n.d.	n.d.
103	AA 023	NoCq	NoCq	NoCq	24.9	n.d.	n.d.
104	BB 023	NoCq	NoCq	NoCq	25.5	n.d.	n.d.
105	CC 023	NoCq	NoCq	NoCq	25.4	n.d.	n.d.
106	DD 023	NoCq	NoCq	NoCq	25.6	n.d.	n.d.
107	EE 023	NoCq	NoCq	NoCq	25.7	n.d.	n.d.
108	FF 023	NoCq	NoCq	NoCq	25.4	n.d.	n.d.
109	AA 024	NoCq	NoCq	NoCq	26.2	n.d.	n.d.
110	BB 024	NoCq	NoCq	NoCq	26.7	n.d.	n.d.
111	CC 024	NoCq	NoCq	NoCq	26.3	n.d.	n.d.
112	DD 024	NoCq	NoCq	NoCq	25.3	n.d.	n.d.
113	EE 024	NoCq	NoCq	NoCq	25.3	n.d.	n.d.
114	FF 024	NoCq	NoCq	NoCq	25.3	n.d.	n.d.
115	AA 025	NoCq	NoCq	NoCq	25.9	n.d.	n.d.
116	BB 025	NoCq	NoCq	NoCq	24.9	n.d.	n.d.
117	CC 025	NoCq	NoCq	NoCq	25.3	n.d.	n.d.
118	DD 025	NoCq	NoCq	NoCq	24.6	n.d.	n.d.
119	EE 025	NoCq	NoCq	NoCq	25.1	n.d.	n.d.
120	FF 025	NoCq	NoCq	NoCq	25.5	n.d.	n.d.
121	AA 026	NoCq	NoCq	NoCq	24.8	n.d.	n.d.
122	BB 026	NoCq	NoCq	NoCq	25.7	n.d.	n.d.
123	CC 026	NoCq	NoCq	NoCq	25.7	n.d.	n.d.
124	DD 026	NoCq	NoCq	NoCq	26.1	n.d.	n.d.

		multiplex RT-qPCR				3D-OIE	
FLI ID	TPI ID	FMDV Cq	BVDV Cq	RVFV Cq	β-actin Cq	FMDV Cq	β-actin Cq
125	EE 026	NoCq	NoCq	NoCq	26.8	n.d.	n.d.
126	FF 026	NoCq	NoCq	NoCq	24.3	n.d.	n.d.
127	AA 027	NoCq	NoCq	NoCq	25.2	n.d.	n.d.
128	BB 027	NoCq	NoCq	NoCq	24.9	n.d.	n.d.
129	CC 027	NoCq	NoCq	NoCq	24.7	n.d.	n.d.
130	DD 027	NoCq	NoCq	NoCq	24.8	n.d.	n.d.
131	EE 027	NoCq	NoCq	NoCq	25.2	n.d.	n.d.
132	FF 027	NoCq	NoCq	NoCq	24.4	n.d.	n.d.
133	AA 028	NoCq	NoCq	NoCq	25.3	n.d.	n.d.
134	BB 028	NoCq	NoCq	NoCq	26.5	n.d.	n.d.
135	CC 028	NoCq	NoCq	NoCq	25.0	n.d.	n.d.
136	DD 028	NoCq	NoCq	NoCq	24.5	n.d.	n.d.
137	EE 028	NoCq	NoCq	NoCq	24.1	n.d.	n.d.
138	FF 028	NoCq	NoCq	NoCq	25.1	n.d.	n.d.
139	AA 029	NoCq	NoCq	NoCq	26.8	n.d.	n.d.
140	BB 029	NoCq	NoCq	NoCq	26.2	n.d.	n.d.
141	CC 029	NoCq	NoCq	NoCq	24.3	n.d.	n.d.
142	DD 029	NoCq	NoCq	NoCq	25.4	n.d.	n.d.
143	EE 029	NoCq	NoCq	NoCq	25.5	n.d.	n.d.
144	FF 029	NoCq	NoCq	NoCq	25.7	n.d.	n.d.
145	AA 030	NoCq	NoCq	NoCq	24.9	n.d.	n.d.
146	BB 030	NoCq	NoCq	NoCq	25.1	n.d.	n.d.
147	CC 030	NoCq	NoCq	NoCq	24.7	n.d.	n.d.
148	DD 030	NoCq	NoCq	NoCq	24.2	n.d.	n.d.
149	EE 030	NoCq	NoCq	NoCq	24.4	n.d.	n.d.
150	FF 030	NoCq	NoCq	NoCq	24.7	n.d.	n.d.
151	AA 031	NoCq	NoCq	NoCq	25.1	n.d.	n.d.
152	BB 031	NoCq	NoCq	NoCq	26.2	n.d.	n.d.
153	CC 031	NoCq	NoCq	NoCq	25.9	n.d.	n.d.
154	DD 031	NoCq	NoCq	NoCq	26.2	n.d.	n.d.
155	EE 031	NoCq	NoCq	NoCq	26.0	n.d.	n.d.
156	FF 031	NoCq	NoCq	NoCq	27.6	n.d.	n.d.
157	AA 032	NoCq	NoCq	NoCq	26.4	n.d.	n.d.
158	BB 032	NoCq	NoCq	NoCq	26.7	n.d.	n.d.
159	CC 032	NoCq	NoCq	NoCq	26.6	n.d.	n.d.
160	DD 032	NoCq	NoCq	NoCq	26.5	n.d.	n.d.
161	EE 032	NoCq	NoCq	NoCq	26.4	n.d.	n.d.
162	FF 032	NoCq	NoCq	NoCq	26.4	n.d.	n.d.
163	AA 033	NoCq	NoCq	NoCq	27.1	n.d.	n.d.
164	BB 033	NoCq	NoCq	NoCq	28.0	n.d.	n.d.
165	CC 033	NoCq	NoCq	NoCq	27.2	n.d.	n.d.
166	DD 033	NoCq	NoCq	NoCq	27.0	n.d.	n.d.
167	EE 033	NoCq	NoCq	NoCq	26.5	n.d.	n.d.
168	FF 033	NoCq	NoCq	NoCq	26.3	n.d.	n.d.
169	AA 034	NoCq	NoCq	NoCq	26.4	n.d.	n.d.
170	BB 034	NoCq	NoCq	NoCq	26.8	n.d.	n.d.
171	CC 034	NoCq	NoCq	NoCq	25.9	n.d.	n.d.
172	DD 034	NoCq	NoCq	NoCq	27.4	n.d.	n.d.
173	EE 034	NoCq	NoCq	NoCq	27.5	n.d.	n.d.
174	FF 034	NoCq	NoCq	NoCq	27.3	n.d.	n.d.
175	AA 035	NoCq	NoCq	NoCq	26.3	n.d.	n.d.
176	BB 035	NoCq	NoCq	NoCq	26.3	n.d.	n.d.
177	CC 035	NoCq	NoCq	NoCq	26.1	n.d.	n.d.
178	DD 035	NoCq	NoCq	NoCq	26.8	n.d.	n.d.
179	EE 035	NoCq	NoCq	NoCq	26.1	n.d.	n.d.

		multiplex RT-qPCR				3D-OIE	
FLI ID	TPI ID	FMDV Cq	BVDV Cq	RVFV Cq	β-actin Cq	FMDV Cq	β-actin Cq
180	FF 035	NoCq	NoCq	NoCq	27.6	n.d.	n.d.
181	AA 036	NoCq	NoCq	NoCq	26.2	n.d.	n.d.
182	BB 036	NoCq	NoCq	NoCq	27.1	n.d.	n.d.
183	CC 036	NoCq	NoCq	NoCq	27.0	n.d.	n.d.
185	EE 036	NoCq	NoCq	NoCq	25.9	n.d.	n.d.
186	FF 036	NoCq	NoCq	NoCq	27.0	n.d.	n.d.
187	AA 037	NoCq	NoCq	NoCq	26.3	n.d.	n.d.
188	BB 037	NoCq	NoCq	NoCq	27.2	n.d.	n.d.
189	CC 037	NoCq	NoCq	NoCq	26.3	n.d.	n.d.
190	DD 037	NoCq	NoCq	NoCq	26.2	n.d.	n.d.
191	EE 037	NoCq	NoCq	NoCq	26.4	n.d.	n.d.
192	FF 037	NoCq	NoCq	NoCq	26.1	n.d.	n.d.
193	AA 038	NoCq	NoCq	NoCq	26.4	n.d.	n.d.
194	BB 038	NoCq	NoCq	NoCq	26.9	n.d.	n.d.
195	CC 038	NoCq	NoCq	NoCq	26.1	n.d.	n.d.
196	DD 038	NoCq	NoCq	NoCq	25.6	n.d.	n.d.
197	EE 038	NoCq	NoCq	NoCq	25.7	n.d.	n.d.
198	FF 038	NoCq	NoCq	NoCq	26.7	n.d.	n.d.
199	AA 039	NoCq	NoCq	NoCq	26.4	n.d.	n.d.
200	BB 039	NoCq	NoCq	NoCq	27.1	n.d.	n.d.
201	CC 039	NoCq	NoCq	NoCq	26.2	n.d.	n.d.
202	DD 039	NoCq	NoCq	NoCq	25.8	n.d.	n.d.
203	EE 039	NoCq	NoCq	NoCq	26.3	n.d.	n.d.
204	FF 039	NoCq	NoCq	NoCq	26.6	n.d.	n.d.
205	AA 040	NoCq	NoCq	NoCq	26.6	n.d.	n.d.
206	BB 040	NoCq	NoCq	NoCq	26.5	n.d.	n.d.
207	CC 040	NoCq	NoCq	NoCq	25.7	n.d.	n.d.
209	EE 040	NoCq	NoCq	NoCq	26.3	n.d.	n.d.
210	FF 040	NoCq	NoCq	NoCq	26.1	n.d.	n.d.
211	AA 041	NoCq	NoCq	NoCq	26.2	n.d.	n.d.
212	BB 041	NoCq	NoCq	NoCq	27.1	n.d.	n.d.
213	DD 041	NoCq	NoCq	NoCq	29.6	n.d.	n.d.
214	EE 041	NoCq	NoCq	NoCq	28.2	n.d.	n.d.
215	FF 041	NoCq	NoCq	NoCq	27.1	n.d.	n.d.
216	AA 042	NoCq	NoCq	NoCq	26.7	n.d.	n.d.
217	BB 042	NoCq	NoCq	NoCq	26.8	n.d.	n.d.
218	DD 042	NoCq	NoCq	NoCq	26.1	n.d.	n.d.
219	EE 042	NoCq	NoCq	NoCq	26.3	n.d.	n.d.
220	FF 042	NoCq	NoCq	NoCq	26.7	n.d.	n.d.
221	AA 043	NoCq	NoCq	NoCq	27.7	n.d.	n.d.
222	BB 043	NoCq	NoCq	NoCq	25.9	n.d.	n.d.
223	DD 043	NoCq	NoCq	NoCq	26.1	n.d.	n.d.
224	EE 043	NoCq	NoCq	NoCq	26.5	n.d.	n.d.
225	FF 043	NoCq	NoCq	NoCq	26.0	n.d.	n.d.
226	AA 044	NoCq	NoCq	NoCq	26.1	n.d.	n.d.
227	BB 044	NoCq	NoCq	NoCq	26.2	n.d.	n.d.
228	DD 044	NoCq	NoCq	NoCq	25.9	n.d.	n.d.
229	EE 044	NoCq	NoCq	NoCq	30.2	n.d.	n.d.
230	FF 044	NoCq	NoCq	NoCq	26.2	n.d.	n.d.
231	AA 045	NoCq	NoCq	NoCq	26.6	n.d.	n.d.
232	BB 045	NoCq	NoCq	NoCq	26.4	n.d.	n.d.
233	DD 045	NoCq	NoCq	NoCq	26.9	n.d.	n.d.
234	EE 045	NoCq	NoCq	NoCq	26.9	n.d.	n.d.
235	FF 045	NoCq	NoCq	NoCq	27.2	n.d.	n.d.
NL-3	-	NoCq	NoCq	NoCq	30.5	n.d.	n.d.

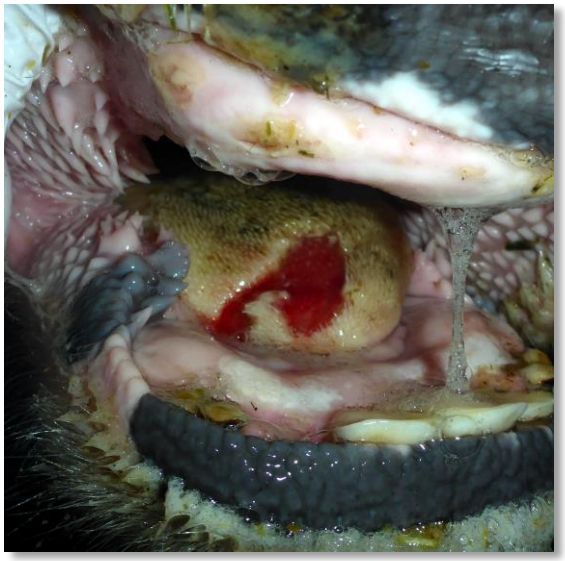
“bulk milk testing”

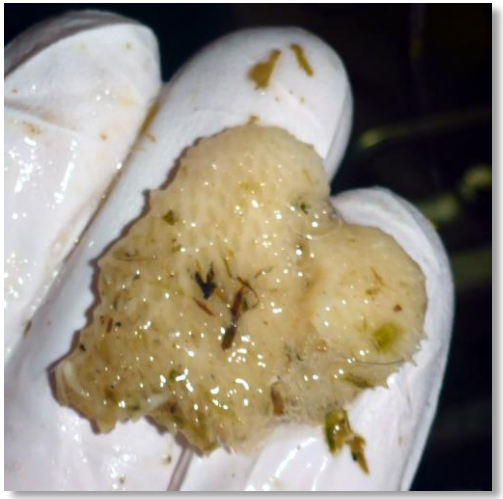
	cow 530	cow 542	cow 790
neat	27.2	26.2	21.8



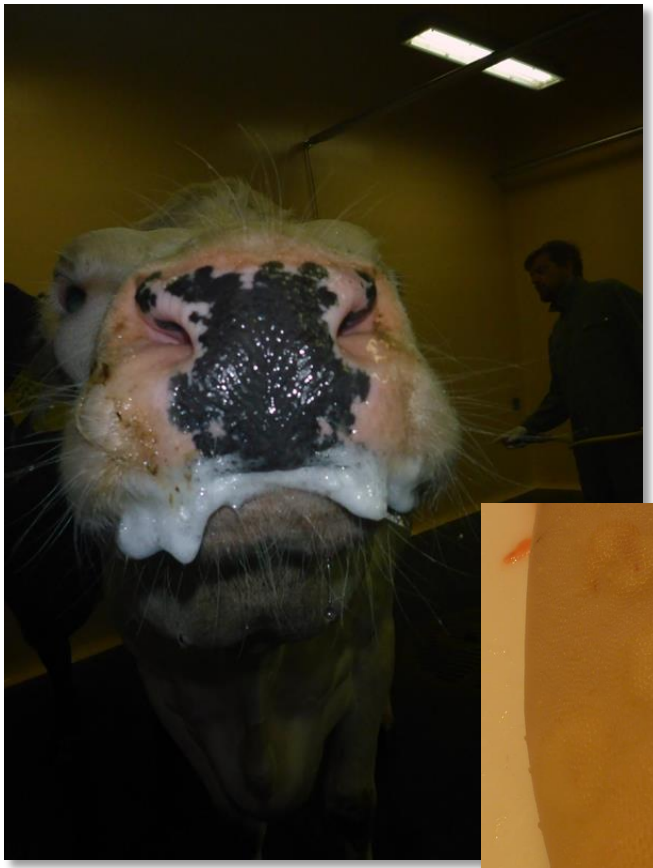
Conclusions I

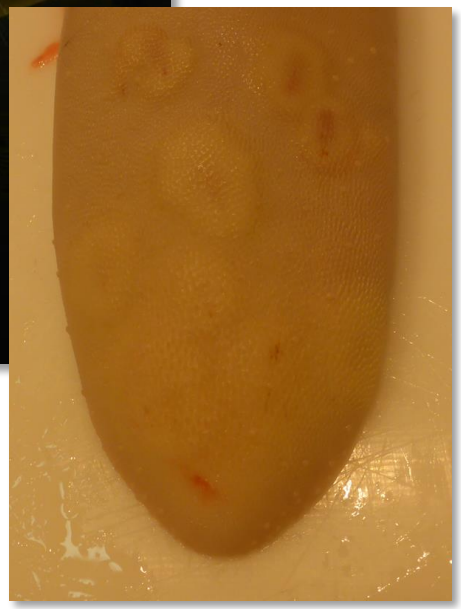
- FMDV RNA is easily detectable in milk
- Detection in milk outlasts detection in serum and other secretions
- Pre-treatment of milk (phase separation) is not necessary
- Sensitivity of the multiplex assay currently is not sufficient for early outbreak detection by bulk milk testing
- The multiplex assay requires further validation for BVDV and RVFV with *ex vivo* samples



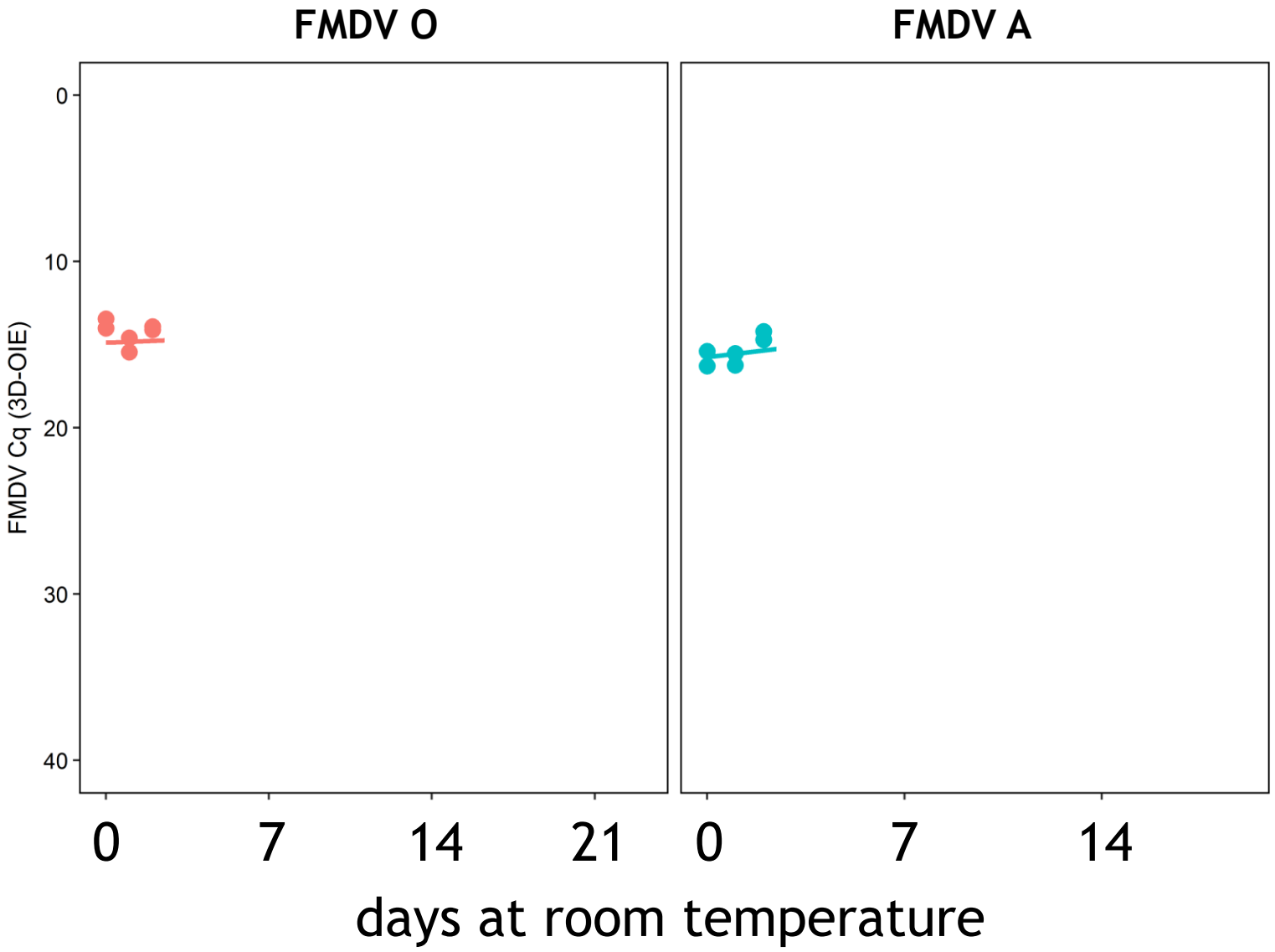


530, 5 dpi
A/IRN/22/2015





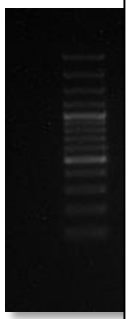
506, 1 dpi
O/FRA/1/2001


FMDV O
FMDV A
FMDV Cq (3D-OIE)
0
10
20
30
40
0
7
14
21
0
7
14
days at room temperature

Journal of Virological Methods 246 (2017) 58–64

Contents lists available at ScienceDirect

Journal of Virological Methods



THE Pirbright INSTITUTE

OIE/FAO Foot-and-Mouth Disease Reference Laboratories Network

BBSRC bioscience for the future

FMDV Toolbox

ods

viromet

sequencing

disease viruses

CrossMark

sample

Inf 1-18 epithelium 1 day RT-A

Inf 1-18 epithelium 1 day RT-B

Inf 1-18 epithelium 19 days RT-

Inf 1-18 epithelium 19 days RT-

Inf 3-18 epithelium 1 day RT-A

Inf 3-18 epithelium 1 day RT-B

Inf 3-18 epithelium 23 days RT-

Inf 3-18 epithelium 23 days RT-

Sequence ID	Genetic distance (% nucleotide diffs)	Country	Year	Similar sequences
IRN/8/2015	0.352	Iran	2015	3
IRN/21/2015	0.704	Iran	2015	0
ARM/2/2015	0.704	Armenia	2015	0
IRN/12/2015	0.704	Iran	2015	1
IRN/14/2015	1.056	Iran	2015	10
IRN/8/2016	1.056	Iran	2016	1
TUR/1218/2016.769	1.408	Turkey	2016	0
SAU/5/2015	1.408	Saudi Arabia	2015	0
TUR/1225/2016.769	1.408	Turkey	2016	0
SAU/21/2016	1.408	Saudi Arabia	2016	2
IRN/39/2016	1.408	Iran	2016	0
SAU/7/2015	1.408	Saudi Arabia	2015	1
SAU/14/2015	1.408	Saudi Arabia	2015	3
SAU/1/2015	1.408	Saudi Arabia	2015	3
IRN/23/2016	1.408	Iran	2016	2
PD78/IND/2015	1.761	India	2015	0
SAU/3/2015	1.761	Saudi Arabia	2015	1
TUR/1008/2016.500	1.761	Turkey	2016	0

For further information about the sequences above and to include them in publications, contact *fmdvtools@pirbright.ac.uk*.

Conclusions II

- FMDV RNA is easily detectable in vesicular epithelium that has been stored at room temperature without buffer for several weeks
- viral RNA integrity is sufficient for VP1 sequencing and transfection

→ **If you're dealing with a real case, sample quality is secondary!**

This study was funded by the EuFMD Fund for Applied Research.

FRIEDRICH-LOEFFLER-INSTITUT
since 1910
FLI
Bundesforschungsinstitut für Tiergesundheit
Federal Research Institute for Animal Health

FMDV adsorbed to GenoTube swabs remains infectious for ≥2 hours at 100 °C and for ≥6 months at room temperature

Michael Eschbaumer, National Reference Laboratory for Foot-and-Mouth Disease, Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany. E-Mail: michael.eschbaumer@fli.de

Introduction

In previous studies, self-drying foam swabs (GenoTube Livestock, Thermo Fisher Scientific) were successfully used to collect samples for the diagnosis of classical and African swine fever (Petrov *et al.*, 2014, Vet Microbiol 173[3-4]:360-5). Here, we evaluated them as an alternative method for the collection and transport of foot-and-mouth disease virus (FMDV) samples or cultures. The study covered two aspects: the retention of FMDV infectivity on swabs stored at room temperature and its inactivation by heating.

Residual infectivity after heat treatment of GenoTube swabs soaked with FMDV

Three aliquots of a high-titer stock of FMDV A IRN/8/2015 were prepared in microcentrifuge tubes. GenoTubes were dipped in the virus suspension and left to dry in a biosafety cabinet. The fully dried swabs were placed for 120 minutes in a forced-air laboratory oven pre-heated to 100 °C. A second set of swabs was prepared in the same way, but not heated. These swabs and the dipping tubes were closed and stored at room temperature.

After heating, the swabs from the oven were allowed to cool to room temperature. All swabs were then processed together. Half of the absorbent foam at the tip of each swab was cut off and added to a microcentrifuge tube with serum-free cell culture media. The tubes were vortexed thoroughly, incubated at room temperature for 5 minutes and centrifuged briefly. The swab supernatants and the virus from the dipping tubes were titrated on LFBK-αVb6 cells in 96-well plates (LaRocco *et al.*, 2013, J Clin Microbiol 51[6]:1714-20). After three days of incubation at 37 °C, cytopathic effect (CPE) on the plates was evaluated microscopically. The experiment was performed twice with three replicates per run. The results were similar between both runs, therefore only the results of the second run are presented.

Heat transfer to the interior of GenoTube swab tubes

The GenoTube swabs are shipped in thick-walled and tightly sealed plastic tubes. To assess the time required for the transfer of externally applied heat to the swab itself, a thermocouple was placed inside a sealed swab tube and the tube was placed in a forced-air laboratory oven preheated to 100 °C.

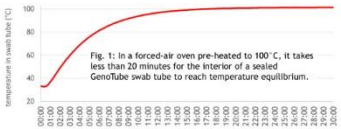


Fig. 1: In a forced-air oven pre-heated to 100 °C, it takes less than 20 minutes for the interior of a sealed GenoTube swab tube to reach temperature equilibrium.

Confirmation of specificity of observed CPE in LFBK-αVB6 cells

2A virus stock in dipping tube

CPE	tube 1	tube 2	tube 3
10^-1	+ + +	+ + +	+ + +
10^-2	+ + +	+ + +	+ + +
10^-3	+ + +	+ + +	+ + +
10^-4	+ + +	+ + +	+ + +
10^-5	+ + +	+ + +	+ + +
10^-6	+ + +	+ + +	+ + +
10^-7	- - -	- - +	+ + +
cell ctrl	- - -	- - -	- - -

2B virus adsorbed to GenoTube, not heated

CPE	swab 1	swab 2	swab 3
10^-1	+ + +	+ + +	+ + +
10^-2	+ + +	+ + +	+ + +
10^-3	+ + +	+ + +	+ + +
10^-4	+ + +	+ + +	+ + +
10^-5	- - +	+ + +	+ + +
10^-6	+ - -	- - -	- - +
10^-7	- - -	- - -	- - -
cell ctrl	- - -	- - -	- - -

2D virus adsorbed to GenoTube, not heated

OD	swab 1	swab 2	swab 3
10^-1	1.86 2.02 1.99	2.06 2.03 2.01	1.99 1.96 2.18
10^-2	0.89 0.89 0.89	0.98 1.00 0.99	0.89 0.88 0.93
10^-3	0.57 0.56 0.53	0.56 0.53 0.54	0.56 0.54 0.59
10^-4	0.55 0.60 0.56	0.46 0.72 0.75	0.57 0.60 0.56
10^-5	0.07 0.06 1.25	1.01 1.01 0.84	0.64 0.77 1.27
10^-6	1.14 0.06 0.71	0.06 0.07 0.06	0.07 0.06 0.86
10^-7	0.07 0.06 0.06	0.07 0.06 0.07	0.07 0.07 0.07
cell ctrl	0.07 0.07 0.07	0.07 0.07 0.07	0.07 0.07 0.07

The culture supernatants from the virus titration plates 2B and 2C were tested in a standard FMDV serotype A antigen ELISA.

Fig. 2D/E (left): Optical density (OD) at 492 nm wavelength of FMDV serotype A antigen ELISA plates. The positive cut-off was set at three times the mean OD of the negative controls in the bottom row (0.22). Wells with optical densities above the cut-off are marked in red.

All wells that had shown CPE contained high amounts of FMDV serotype A antigen, confirming the presence of infectious FMDV in the heat-treated swabs.

Fig. 2: CPE on LFBK-αVB6 plates after 3 days. Wells with CPE are marked in red. To confirm cell viability, the bottom row of each plate contained cells that were not inoculated.

	virus stock	no heat	100°C for 120 min
swab 1	6.5	5.5	2.8
swab 2	6.8	5.5	2.5
swab 3	7.2	5.8	2.8
average	6.8	5.6	2.7
reduction		1.2	2.9

Tab. 1: Overview of FMDV titers with and without heat treatment. All titers are $\log_{10}$ 50% tissue culture infectious doses on LFBK-αVB6 cells per 100 µl.

2C adsorbed, then heated to 100°C for 120 minutes

CPE	swab 1	swab 2	swab 3
10^-1	+ + +	+ + +	+ + +
10^-2	+ + +	+ + +	+ + +
10^-3	+ - -	- - -	+ - -
10^-4	- - -	- - -	- - -
10^-5	- - -	- - -	- - -
10^-6	- - -	- - -	- - -
10^-7	- - -	- - -	- - -
cell ctrl	- - -	- - -	- - -

2E adsorbed, then heated to 100°C for 120 minutes

OD	swab 1	swab 2	swab 3
10^-1	1.75 1.57 1.56	1.61 1.71 1.64	1.44 1.52 1.58
10^-2	0.86 0.70 0.68	1.45 0.79 1.13	0.66 0.67 0.81
10^-3	1.06 0.07 0.07	0.07 0.07 0.08	0.90 0.07 0.07
10^-4	0.07 0.07 0.07	0.07 0.07 0.08	0.07 0.07 0.08
10^-5	0.07 0.08 0.08	0.07 0.07 0.08	0.08 0.07 0.07
10^-6	0.07 0.07 0.06	0.06 0.07 0.07	0.07 0.07 0.08
10^-7	0.07 0.07 0.07	0.08 0.07 0.08	0.07 0.07 0.07
cell ctrl	0.07 0.08 0.08	0.07 0.08 0.08	0.09 0.06 0.07

FMDV A IRN/8/2015 adsorbed to GenoTube Livestock swabs was highly resistant to heating and retained its infectivity at room temperature for a long time. The dried swabs must be shipped under IATA regulations for infectious substances. However, they do not require refrigeration or dry ice, making them a convenient and low-cost alternative to the shipping of frozen liquid samples.

Long-term storage at room temperature of GenoTubes soaked with FMDV

For the long-term storage test, five sets of three GenoTube swabs were soaked with FMDV and dried as described above. One set of swabs was processed immediately after drying, the others were placed in tightly sealed containers (1.5L Plastic Biojars, Air Sea Containers Ltd.) and stored on a shelf in the laboratory. They underwent normal diurnal temperature variations, but generally remained between 20 °C and 23 °C for the duration of the experiment. One set of GenoTubes was taken off the shelf after 1, 4, 12 and 24 weeks. The swabs were processed as described above and the supernatants stored at -80 °C. All supernatants were titrated together at the end of the experiment at 24 weeks.

Fig. 4A-E: Titration of swab supernatants on LFBK-αVB6 cells. Wells with CPE after 3 days are marked in red. To confirm cell viability, the bottom row of each plate contained cells that were not inoculated.

4A GenoTubes processed after drying ("0 weeks")

CPE	swab 1	swab 2	swab 3
10^-1	+ + +	+ + +	+ + +
10^-2	+ + +	+ + +	+ + +
10^-3	+ + +	+ + +	+ + +
10^-4	+ + +	+ + +	+ + +
10^-5	+ + +	+ + +	+ + +
10^-6	- - -	+ - -	- - -
10^-7	- - -	+ - -	- + -
cell ctrl	- - -	- - -	- - -

4B GenoTubes incubated at RT for 1 week

CPE	swab 1	swab 2	swab 3
10^-1	+ + +	+ + +	+ + +
10^-2	+ + +	+ + +	+ + +
10^-3	+ + +	+ + +	+ + +
10^-4	- + -	+ + +	+ + +
10^-5	- - -	- - -	- - -
10^-6	- - -	- - -	- - -
10^-7	- - -	- - -	- - -
cell ctrl	- - -	- - -	- - -

4C GenoTubes incubated at RT for 4 weeks

CPE	swab 1	swab 2	swab 3
10^-1	+ + +	+ + +	+ + +
10^-2	+ + +	+ + +	+ + +
10^-3	+ + +	+ + +	+ + +
10^-4	- + -	- + -	- + -
10^-5	- - -	- - -	- - -
10^-6	- - -	- - -	- - -
10^-7	- - -	- - -	- - -
cell ctrl	- - -	- - -	- - -

4D GenoTubes incubated at RT for 12 weeks

CPE	swab 1	swab 2	swab 3
10^-1	+ + +	+ + +	+ + +
10^-2	+ + +	+ + +	+ + +
10^-3	+ + +	+ + +	+ + +
10^-4	+ - -	+ - -	+ + -
10^-5	- - -	- - -	- - -
10^-6	- - -	- - -	- - -
10^-7	- - -	- - -	- - -
cell ctrl	- - -	- - -	- - -

4E GenoTubes incubated at RT for 24 weeks

CPE	swab 1	swab 2	swab 3
10^-1	+ + +	+ + +	+ + +
10^-2	+ + +	+ + +	+ + +
10^-3	+ + +	+ + +	+ + +
10^-4	+ + +	+ + +	+ + +
10^-5	- + -	+ + -	- + -
10^-6	- - -	- - -	- - -
10^-7	- - -	- - -	- - -
cell ctrl	- - -	- - -	- - -

4F GenoTubes incubated at room temperature for 24 weeks

OD	swab 1	swab 2	swab 3
10^-1	0.74 0.73 0.74	0.77 0.77 0.75	0.74 0.77 0.92
10^-2	0.52 0.62 0.58	0.58 0.69 0.67	0.59 0.69 0.65
10^-3	0.57 0.77 0.71	0.75 0.64 0.66	0.60 0.59 0.58
10^-4	0.73 0.61 0.58	0.06 0.26 0.65	0.75 0.06 0.65
10^-5	0.06 0.63 0.06	0.06 0.46 0.58	0.06 0.05 0.05
10^-6	0.06 0.06 0.05	0.05 0.05 0.05	0.05 0.05 0.05
10^-7	0.06 0.06 0.06	0.06 0.06 0.06	0.05 0.06 0.05
cell ctrl	0.05 0.06 0.06	0.05 0.06 0.06	0.06 0.06 0.06

virus eluted from GenoTubes stored at RT

	0 wk	1 wk	4 wk	12 wk	24 wk
swab 1	5.5	4.0	3.8	3.8	4.5
swab 2	6.3	4.3	4.0	4.0	4.8
swab 3	6.0	4.5	3.8	4.3	5.0
average	5.9	4.3	3.8	4.0	4.8

Tab. 2 (left): Overview of FMDV titers over the course of the storage experiment. All titers shown are $\log_{10}$ 50% tissue culture infectious doses on LFBK-αVB6 cells per 100 µl.

Virus titer decreased by about 2 $\log_{10}$ during the first week, but then did not decrease further over the course of 3 months of storage at room temperature.

Fig. 4F: Optical density readings of FMDV serotype A antigen ELISA plate at 492 nm wavelength. The positive cut-off was set at three times the mean OD of the negative controls in the bottom row (0.17). Wells with ODs above the cut-off are marked in red.

All wells of plate 4E that had shown CPE contained high amounts of FMDV serotype A antigen, confirming the presence of infectious FMDV in the sample that had been stored at room temperature for 6 months.

This study was supported by the EuFMD Fund for Applied Research.

Thank you for your attention!

Questions?

FRIEDRICH-LOEFFLER-INSTITUT
seit 1910
FLI
Bundesforschungsinstitut für Tiergesundheit
Federal Research Institute for Animal Health