RESEARCH REPORT
SWINE HEALTH

Title: Evaluation of meat juice as sample matrix for the detection of foot-and-mouth disease virus (FMDV) antigen and nucleic acids as well as antibodies to FMDV; and comparison of 3 real-time reverse transcription polymerase chain reaction assays for FMDV in swine oral fluids. **NPB #18-103**

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Industry Summary:

Particular specimens are usually required for foot-and-mouth disease virus (FMDV) testing. These samples include liquid removed from blisters caused by FMDV (vesicular fluid), pieces of skin recovered from blisters that have burst open (epithelial tags), swabs, serum and other specimens taken from live animals. However, there might be situations when these routine specimens are not available and alternatives may be required. Meat juice (MJ) can be collected from meat that has already been sent to market or from meat imported illegally and tested for FMDV. Meat juice is the liquid that comes out of meat after the meat has been frozen and then defrosted a few times. In this project we wanted to demonstrate that MJ can be used for FMDV detection. Meat was collected from pigs experimentally infected with FMDV. Meat juice was recovered from this meat, ribonucleic acid (RNA) was extracted from the MJ and tested for the presence of FMDV by real-time reverse transcriptase polymerase chain reactions (RRT-PCR) using the National Centre for Foreign Animal Disease (NCFAD) protocol in parallel with the US National Animal Health Laboratory Network (NAHLN) FMDV RRT-PCR and TetraCore (commercial) FMDV RRT-PCR detection kit. Meat juice was also tested by lateral flow strip tests (LFST) for detecting specific proteins (antigens) found on the surface of FMDV. All 3 RRT-PCR assays detected FMDV RNA in MJ from pigs infected with either FMDV serotype A, O, SAT2 or ASIA1. In all cases, FMDV genome detection in sera was short-lived compared to MJ which lasted up to 21 days after infection in some cases. LFSTs detected FMDV antigen in MJ in the early days following infection (days 1 – 9). Furthermore, this study assessed MJ for detection of antibodies to proteins on the surface of FMDV using tests available at the NCFAD. Antibodies to FMDV were detected in MJ from experimentally infected pigs in similar patterns to antibody detection in sera tested in parallel. All this data shows that MJ is a good sample type for FMDV nucleic acid and antigen detection, as well as detection of antibodies to proteins on the surface of FMDV. Therefore, in the absence of traditional samples, MJ can be used for FMDV testing. In addition, if animals are slaughtered/euthanized, MJ can be collected alongside other specimens for FMDV testing.

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Key Findings:

- FMDV genome can be detected in meat juice from experimentally infected pigs
- FMDV genome is detected in porcine meat juice for longer periods than in serum
- FMDV antigen can be detected in meat juice by lateral flow strip tests
- Antibodies to FMDV structural proteins can be detected in meat juice at approximately the same time they are detected in serum
- Therefore, meat juice is suitable for FMDV genome and antigen detection, and detection of antibodies to FMDV structural proteins.

Keywords:
Foot-and-mouth disease virus, meat juice, real-time reverse transcription polymerase chain reaction, antigen, antibodies.

Scientific Abstract:
Vesicular fluid, epithelial tags, swabs, serum and other sample types from live animals are routinely used for foot-and-mouth disease virus (FMDV) detection. These samples might not be available in some circumstances and alternative sample types may be required. Meat Juice (MJ) can be collected retroactively from meat for FMDV detection. Meat juice is the exudate recovered after freeze-thaw cycles of skeletal muscle. In this project experiments were performed to demonstrate that MJ can be used for FMDV detection. Skeletal muscle was collected from pigs experimentally infected with FMDV. Meat juice was harvested, ribonucleic acid (RNA) extracted and tested for FMDV genome by real-time reverse transcriptase polymerase chain reactions (RRT-PCR) using the National Centre for Foreign Animal Disease (NCFAD) protocol in parallel with the US National Animal Health Laboratory Network (NAHLN) FMDV RRT-PCR and TetraCore (commercial) FMDV RRT-PCR detection kit. Lateral flow strip test (LFST) was assessed for FMDV antigen detection in MJ. All 3 RRT-PCR assays detected FMDV RNA in MJ from pigs infected with either FMDV serotype A, O, SAT2 or ASIA1. In all cases, FMDV genome detection in sera was short-lived (DPI 1 – 7) compared to MJ which lasted till DPI 21 in some cases. LFSTs detected FMDV antigen in MJ at early DPIs (1 – 9). Furthermore, this study assessed MJ for detection of antibodies to structural proteins (SP) by existing serotype-specific solid phase competitive ELISAs (SPCE). Antibodies to FMDV SP were detected in MJ from experimentally infected pigs in similar trends to antibody detection in sera tested in parallel. The data shows that MJ is a good sample type for FMDV genome and antigen detection, as well as detection of anti-FMDV SP antibodies. Therefore, in the absence of traditional samples, MJ can be used for FMDV testing. In addition, if animals are slaughtered/ euthanized, MJ can be collected alongside other samples for FMDV testing.

Introduction:
Foot-and-mouth disease virus (FMDV), family *Picornaviridae*, causes foot-and-mouth disease (FMD), a highly contagious vesicular disease affecting cloven-hoofed animals such as cattle, sheep, goats and pigs. The disease is endemic or sporadic in many countries worldwide and outbreaks can lead to devastating economic consequences in countries currently free of the disease. FMDV consists of seven antigenically distinct serotypes (O, A, C, Asia-1 and SAT-1, -2 & -3). FMD cannot be clinically differentiated from other vesicular diseases such as swine vesicular disease (SVD), vesicular stomatitis (VS) and vesicular exanthema of swine (Alexandersen et al., 2003). Therefore, rapid and accurate laboratory diagnosis is essential for confirmation of any clinical case.
Samples from individual animals are often required for disease surveillance in populations. Often, when disease prevalence is low, a high number of samples could be required. Additionally, collection of individual samples from live animals requires restraining of animals and use of special equipment. These factors render this approach to disease surveillance costly. Furthermore, the handling and blood collection from animals may pose a risk of further spreading a disease. Alternative cost-effective approaches to disease surveillance are being explored. These include the use of meat juice and oral fluids.

Meat juice is an exudate from meat, specifically skeletal muscle. This sample type has been used for the detection of pathogens and biomarkers of health status in pigs. Meat juice was used to detect classical swine fever virus (CSFV) in experimentally infected pigs. However, virus detection in meat juice was lower compared to serum from the same animals. Nevertheless, this experiment revealed that, in the absence of blood or serum, meat juice can be a useful sample type for CSFV detection (Lohse et al., 2011, Kaden et al., 2009). ASFV genome has also been successfully detected in meat juice from pigs infected with ASFV by real-time PCR assay (McKillen et al., 2010). In addition to virus detection, meat juice has been used for antibody detection in pigs infected with CSFV, influenza A virus, porcine circovirus 2, Aujeszky's disease virus and porcine reproductive and respiratory syndrome virus (PRRSV) as well as bacteria and protozoa (De Lange et al., 2003, Le Potier et al., 1998, Fabisiak et al 2013., Wacheck et al., 2012, Meemken et al., 2014, Kaden et al., 2009). Furthermore, haptoglobin and C-reactive proteins detection in meat juice have been proposed as biomarkers of herd health status (Gómez-Laguna et al., 2010, Gutiérrez et al., 2015). There was, however, no data on the evaluation of meat juice as a diagnostic sample for FMDV. This project filled a significant gap in knowledge on the suitability of this sample type for the detection of FMDV and antibodies to FMDV.

Objectives:
This project evaluated assays for detection of
1. FMDV genome detection in meat juice by real-time reverse transcription polymerase chain reaction (RRT-PCR) using the NCFAD protocol in parallel with the National Animal Health Laboratory Network (NAHLN) FMDV RRT-PCR and TetraCore FMDV RRT-PCR detection kit
2. FMDV antigen in meat juice by a penside (lateral flow) strip test
3. Antibodies to both structural and non-structural proteins of FMDV in meat juice by ELISAs

In addition, oral fluids were collected from these groups of infected pigs to continue assay validation and sharing with other laboratories.

Materials & Methods:

Animal inoculation with FMDV were conducted in containment level 3 at the NCFAD.
Animals:
Pigs aged 5 to 6 weeks were obtained from a local supplier. These animals were examined upon arrival and moved into cubicles. For FMDV serotype A and SAT2 experiments, 36 pigs (6 pigs per cubicle) were

used for each serotype. For FMDV serotype ASIA1 experiment, 24 pigs (6 pigs per cubicle) were used. For FMDV serotype O experiment, 17 animals were used.
Food and water were provided *ad libitum* and the pigs allowed a minimum of 7 days to acclimatise to their new surroundings before the start of each experiment. The animals were visually monitored and their rectal temperatures measured daily.

Viruses:
FMDV serotypes O, A, ASIA1 and SAT2 were selected because of their relatively wide global distribution and the availability at the NCFAD of antigen detection lateral flow strip tests for them. Viruses were produced in appropriate cell cultures and the passage number kept as low as possible. Virus titres were determined in corresponding cell cultures.

Animal inoculation and sampling:
For FMDV serotype A, SAT2 and ASIA1 experiments 3 pigs per cubicle were inoculated intradermally with cell culture supernatants containing 10^3 (A and SAT2) or 10^4 (ASIA1) TCID50 (50% tissue culture infectious dose) of FMDV in the heel bulb of one hind limb. For FMDV serotype O experiment, 17 animals were used. Fifteen were vaccinated with a commercial BEI-inactivated FMD O1 Manisa vaccine and all 17 pigs challenged 10^4 TCID50 of O1 Manisa after 28 days. Meat juice was collected from the pigs showing clinical signs (lesions) and all surviving pigs euthanized at 10 days post-challenge. This was done to assess the potential of detecting antibodies in meat juice from vaccinated animals as well as obtain strong positive meat juice samples for antibody detection ELISAs.
Sampling was done at scheduled days post inoculation (DPI) until the end of the experiment. Blood for serum were collected from the anterior vena cava using a 20 gauge needle. Oral swabs were taken with Dacron tipped swabs. Collection of oral fluids samples were performed as previously described (Pricket et al, 2008, Senthilkumaran et al, 2016, 2017). Euthanasia of pigs for meat juice collection was done at scheduled DPI or whenever a pig attained a humane endpoint, at least 4 pigs per time point. Meat (muscle tissues) from the quadriceps muscles were collected from each pig, sectioned into approximately 2 cm^3 pieces and transferred into plastic bags for freezing at –70°C. The meat samples were subsequently thawed at 4°C, and meat juice (exudate) collected into cryovials for testing. Sections of lymph nodes (submandibular, prescapular and popliteal), tonsils, heart were collected in Universal Transport Media for virus detection.

Real-time RT-PCR assay:
The MagMax™ Viral RNA Isolation kit (Ambion) were used for RNA extraction from 55µl of each sample according to manufacturer's protocol [Manual: MagMax-96 Viral RNA Isolation Kit (AM1836), Version: 2013-October-08]. The MagMAX™ Express-96 Instrument was used for purification using a Deep Well Magnetic Particle Processor. The RNA was eluted into 90µl of Elution buffer. Extracted RNA was then tested on 3 FMDV RRT-PCR assays. The NCFAD, NAHLN and TetraCore assays all target the highly conserved 3D gene. However, the primers and probe of the TetraCore assay are proprietary. The NCFAD FMDV RRT-PCR was carried out according to a published protocol (Moniwa et al, 2007; Senthilkumaran et al, 2017). This RRT-PCR uses primers and probe that specifically target an 88bp product (Moniwa et al, 2007). The NAHLN FMDV RRT-PCR was performed according the protocol obtained from USDA/APHIS, Plum Island Animal Disease Center. The TetraCore, Inc. FMDV RRT-PCR used the commercially available FMDV 2.0 reagents with inhibition control (TC-9092-64). The assay was performed as previously described (Howson et al, 2017). Briefly, lyophilized reagent was resuspended in 20 uL of resuspension buffer followed by the addition of 5 uL of RNA. The cycling conditions of 48°C for 15 min, 95°C for 2 min, followed by 45 cycles of 95°C for 10 s and 60°C for 40 s were used on ABI 7500. Data was generated using the RNA extracted with the MagMax™ Viral RNA Isolation kit.

Rapid FMDV antigen detection by lateral flow strip test:
In-house lateral flow strip tests for rapid detection of FMDV serotypes O, A, Asia 1 and SAT 2 were recently developed at the NCFAD (Yang et al, 2013). Meat juice samples from FMDV infected pigs were tested on these devices.

Serological assays:
Antibodies against the non-structural protein (NSP) of FMDV were tested by ELISAs. The NCFAD in-house competitive ELISA (cELISA) uses a recombinant 3ABC protein as antigen and antibodies in serum compete with an anti-3B monoclonal antibody for specific epitopes on the recombinant protein (Clavijo et al, 2004). Furthermore, meat juice were tested on the solid phase competitive ELISA (SPCE) for detection of antibodies to the structural proteins of each serotype of FMDV according to published protocol (Moniwa et al, 2012). Results from meat juice were compared with corresponding sera.

Results:
FMDV genome detection in meat juice by real-time reverse transcription polymerase chain reaction (RRT-PCR) using the NCFAD protocol in parallel with the National Animal Health Laboratory Network (NAHLN) FMDV RRT-PCR and TETRACORE FMDV RRT-PCR detection kit

FMDV A22 IRQ 24/64 experiment
Part of this data has been published " Detection of Foot-and-Mouth Disease Virus in Swine Meat Juice. Yeo S, Yang M, Nyachoti M, Rauh R, Callahan JD, **Nfon C.** Pathogens. 2020 May 29;9(6):424. doi: 10.3390/pathogens9060424.Pathogens. 2020. PMID: 32485851

In the FMDV A22 IRQ 24/64 experiment, FMDV genome was detected in meat juice (MJ) as early as one day post infection (DPI) to as late as DPI 21. The NAHLN and TetraCore RRT-PCRs were relatively more sensitive than the NCFAD RRT-PCR for the detection of FMDV RNA (Figure 1) especially at the early (DPI 1) and late (DPI 21) time points post infection. Specifically, the NALHN and TetraCore assays detected FMDV RNA at DPI 1 and 21 but all MJ samples at these time points were negative by the NCFAD RRT-PCR assay. Viremia based on FMDV RNA detection in sera with any of the RRT-PCR assays started at DPI 1. Viremia was cleared within 4 – 5 days after first detection (Figure 1).

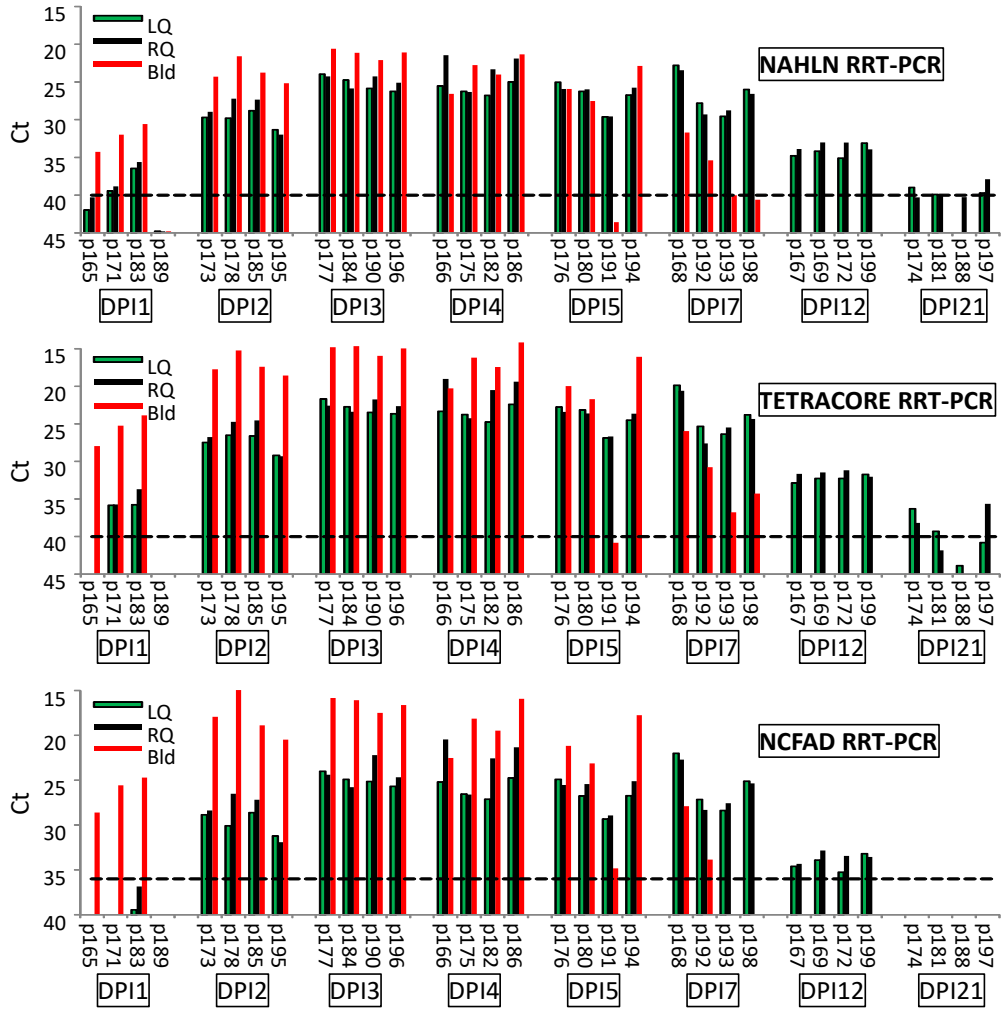


Figure 1: Detection of FMDV in meat juice and serum by 3 RRT-PCRs. Six groups of 6 pigs/group/room were used in this experiment. Three pigs per group were directly inoculated with 1000 $TCID_{50}$ of FMDV A22 Iraq by intradermal inoculation into the heel bulb. Starting at 1 day post infection (DPI) four pigs with the most severe clinical disease were euthanized and quadriceps muscle taken for meat juice collection after freeze-thawing. RNA was extracted from the meat juice and tested by each RRT-PCR. NAHLN= National Animal Health Laboratory Network, TETRACORE = commercial supplier of FMDV RRT-PCR kits, NCFAD = National Centre for Foreign Animal Disease. DPI = days post infection

FMDV RNA was also detectable in oral swabs starting at DPI 2 for most pigs. FMDV RNA was still detectable at DPI 21in oral swabs from 2 surviving pigs (Figure 2)

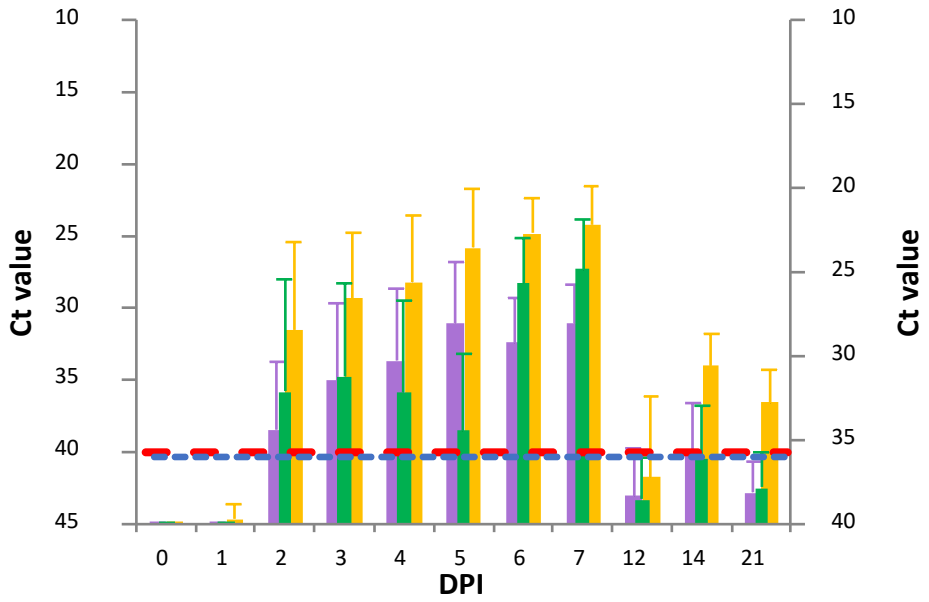


Figure 2: Crossing threshold (Ct) values of Foot-and-mouth disease virus (FMDV) genomic RNA detection in oral swabs from pigs infected with FMDV A22 IRQ 24/64. RNA was extracted using MagMAX™-96 Viral RNA Isolation Kit, and tested with the ABI7500 Fast thermocycler using three different PCR reagent mixes. Threshold for the NCFAD RRT-PCR is 35.99, and 40 for the NAHLN and Tetracore RRT-PCRs. Samples with Ct values below the threshold are considered positive for FMDV

FMDV genome was detected at DPI 1 in oral fluids starting at DPI 1, 2 or 3. The NAHLN and TetraCore RRT-PCRs were once again more sensitive than the NCFAD RRT-PCR for the detection of FMDV RNA (Table 1).

	Day Post Infection (DPI)									
	0	1	2	3	4	5	6	7	12	21
Group 1	Und	Und	Und	29.42	26.97	25.80	24.67	24.37		
	Und	Und	Und	26.46	24.57	23.23	22.90	21.99		
	Und	Und	Und	32.81	29.94	29.50	27.80	27.59		
Group 2	Und	Und	25.36	33.43	25.62	25.20	24.29	28.20		
	Und	38.27	22.70	30.50	23.63	22.69	22.68	26.36		
	Und	Und	29.17	35.99	28.77	28.46	27.68	30.86		
Group 3	Und	Und	24.28	23.57	26.56	27.56	23.22	22.17	35.23	Und
	Und	Und	22.29	21.76	25.27	25.74	22.40	21.34	33.44	Und
	Und	Und	28.12	27.41	30.26	30.61	27.03	26.19	36.51	Und
Group 4	Und	Und	24.52	26.97	23.78	18.84	24.57	29.24	35.89	36.34
	Und	Und	22.55	24.67	22.80	18.25	22.24	27.57	34.13	34.11
	Und	Und	28.78	30.22	28.14	23.33	28.05	31.98	36.72	37.28
Group 5	Und	Und	28.14	26.40	24.61	23.47	21.78	25.79		
	Und	Und	25.85	23.66	23.00	22.25	20.48	24.08		
	Und	Und	31.74	29.99	29.09	27.04	25.41	28.46		
Group 6)	Und	Und	27.86	26.42	34.24	24.96	25.20	23.13		
	Und	Und	25.49	24.21	31.56	23.36	23.47	20.77		
	Und	Und	31.28	29.51	37.00	29.24	28.57	26.41		

Table 1: Crossing Threshold (Ct) for RRT-PCR values of oral fluids from the FMDV A22 IRQ 24/64 experiment. RNA was extracted using MagMAX™-96 Viral RNA Isolation Kit, and tested with the ABI7500 Fast thermocycler using three different PCR reagent mixes. Threshold for the NCFAD RRT-PCR (green) is 35.99, and 40 for the NAHLN (purple) and TetraCore (orange) RRT-PCRs. Samples with Ct values below the threshold are considered positive for FMDV. Grey spaces indicate no sample was collected for that day. Und = undetermined

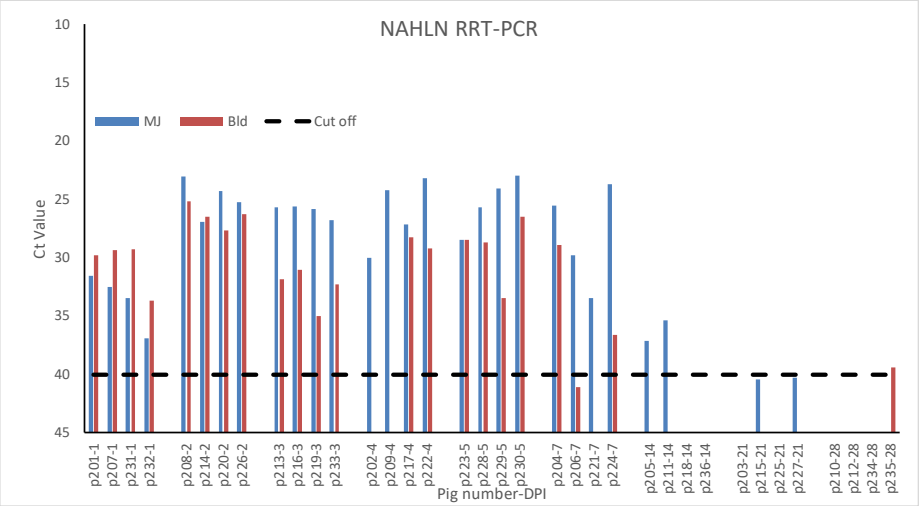
FMDV RNA was detected in the submandibular lymph node from one animal at DPI 1. By DPI 2, all tested lymph nodes (submandibular, prescapular and popliteal) and tonsils were positive for FMDV RNA and stayed positive up to 21 DPI. Overall, the NAHLN and TetraCore RRT-PCRs were more sensitive than the NCFAD RRT-PCR, with the latter failing to detect FMDV in 36 lymph node samples that were positive for FMDV RNA on the former (Table 2).

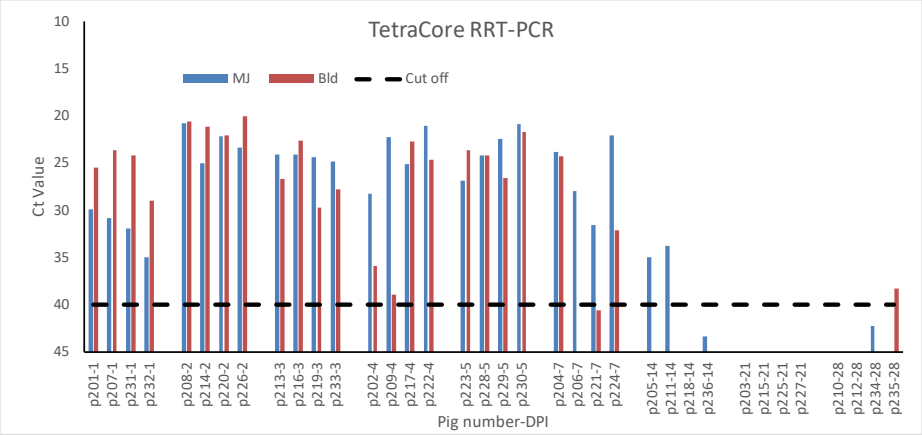
	NAHLN RRT-PCR				TetraCore RRT-PCR				NCFAD RRT-PCR			
Pig # - DPI	Submandibular	Prescapular	Popliteal	Tonsils	Submandibular	Prescapular	Popliteal	Tonsils	Submandibular	Prescapular	Popliteal	Tonsils
p165 - DPI 1	Und	Und	Und	Und	Und	Und	Und	Und	Und	Und	Und	Und
p171 - DPI 1	Und	Und	Und	Und	Und	Und	Und	Und	Und	Und	Und	Und
p183 - DPI 1	39.24	Und	Und	Und	39.22	Und	Und	Und	Und	Und	Und	Und
p189 - DPI 1	Und	Und	Und	Und	Und	Und	Und	Und	Und	Und	Und	Und
p173 - DPI 2	37.27	30.34	30.84	36.31	32.79	27.15	27.15	31.33	Und	29.46	29.67	Und
p178 - DPI 2	30.44	30.40	36.02	34.54	26.92	26.70	29.74	28.97	29.12	28.64	34.88	34.38
p185 - DPI 2	38.03	30.61	37.79	35.80	33.53	27.45	37.96	33.14	Und	29.27	Und	37.21
p195 - DPI 2	37.79	36.98	36.31	38.03	39.58	30.05	33.92	39.71	Und	36.70	Und	Und
p177 - DPI 3	36.21	34.33	35.14	31.68	33.74	31.56	31.65	26.60	Und	31.79	36.33	29.96
p184 - DPI 3	34.21	36.02	34.15		31.44	30.76	31.00		36.56	37.36	33.58	
p190 - DPI 3	36.31	33.56	30.77	33.50	33.07	28.71	26.04	29.71	39.49	32.87	28.27	33.16
p196 - DPI 3	33.57	34.07	30.62	31.86	30.01	28.88	24.32	28.60	34.12	35.30	29.19	30.60
p166 - DPI 4	37.03	31.88	36.78	Und	32.62	26.31	31.13	Und	Und	31.04	Und	Und
p175 - DPI 4	36.80	29.71	33.36	33.34	30.97	25.08	28.60	28.37	Und	28.97	32.77	32.24
p182 - DPI 4	34.47	30.81	36.56	35.55	29.76	25.17	29.64	30.91	35.06	29.49	Und	38.96
p186 - DPI 4	34.98	30.13	31.66	33.74	29.94	26.43	25.07	28.74	36.33	28.90	28.10	32.44
p176 - DPI 5	37.48	32.49	33.02	31.92	31.86	27.71	26.86	27.23	Und	30.63	31.77	30.46
p180 - DPI 5	34.13	32.41	34.02	30.87	29.00	27.81	29.12	25.17	34.20	30.12	33.67	29.27
p191 - DPI 5	35.35	34.73	36.43	29.66	28.59	28.42	28.89	25.40	34.61	32.88	37.59	27.77
p194 - DPI 5	30.96	28.62	30.23	28.35	27.19	22.48	24.00	23.86	30.67	26.73	28.31	26.74
p168 - DPI 7	33.69	33.92	29.18	26.46	28.28	28.49	23.39	23.43	32.65	33.54	27.34	24.33
p192 - DPI 7	31.13	34.87	30.01	25.88	28.84	28.77	23.60	23.23	31.53	32.69	26.93	24.40
p193 - DPI 7	34.95	35.92	Und	31.55	29.01	31.21	Und	28.71	38.81	Und	Und	30.18
p198 - DPI 7	37.85	38.07	36.95	27.34	33.05	35.22	32.02	22.07	Und	Und	Und	25.80
p170 - DPI 8	36.96	33.74	37.34	30.97	30.72	27.98	31.79	27.32	Und	32.57	Und	29.79
p179 - DPI 9	39.50	36.02	33.56	32.52	32.50	29.04	27.13	27.43	Und	Und	35.01	32.10
p187 - DPI 9	31.97	32.37	33.02	33.83	26.53	26.90	25.04	27.87	30.76	32.06	31.23	33.13
p200 - DPI 10	37.47	32.37	36.75	29.40	31.20	24.79	31.38	24.85	Und	30.16	Und	28.34
p167 - DPI 12	39.35	34.72	33.98	30.81	33.40	29.61	28.31	26.82	Und	33.97	33.81	29.89
p169 - DPI 12	31.62	33.80	33.30	30.93	28.53	27.65	26.94	23.67	30.93	32.53	32.24	29.04
p172 - DPI 12	38.76	33.98	Und	35.11	34.29	30.14	Und	30.88	Und	33.66	Und	35.85
p199 - DPI 12	36.24	31.24	35.07	32.47	31.02	27.09	28.33	28.56	Und	30.07	35.21	31.48
p174 - DPI 21	Und	33.63	33.54	30.73	40.76	29.95	27.97	27.23	Und	32.56	33.09	29.35
p181 - DPI 21	30.57	29.08	Und	30.08	28.06	25.58	Und	26.79	29.60	26.94	Und	28.92
p188 - DPI 21	33.33	31.14	31.70	32.14	29.93	26.67	27.03	27.83	32.03	29.07	29.97	30.29
p197 - DPI 21	34.86	33.52	29.20	41.00	33.41	29.90	26.60	40.83	38.57	32.28	28.49	Und

Table 2: Crossing Threshold (Ct) RRT-PCR values of various tissue suspensions (submandibular lymph nodes, prescapular lmph nodes, popliteal lymph nodes, and tonsils) from the FMDV A22 IRQ 24/64 experiment. 10% tissue suspensions were prepared in PBS and clarified by centrifugation. RNA was extracted from tissue suspensions using MagMAX™-96 Viral RNA Isolation Kit, and tested with the ABI7500 Fast thermocycler using three different PCR reagent mixes. Threshold for the NCFAD RRT-PCR (green) is 35.99, and 40 for the NAHLN (purple) and TetraCore (orange) RRT-PCRs. Samples with Ct values below the threshold are considered positive for FMDV. Und = undetermined

FMDV SAT2 Zim 5/81 experiment

In FMDV SAT2 Zim 5/81 experiment, FMDV genome was detected in MJ as early as DPI 1 to as late as DPI 14 (Figure 3). The NAHLN and TetraCore RRT-PCRs were once again more sensitive than the NCFAD RRT-PCR for the detection of FMDV RNA in MJ (Figure 3). Specifically, all assays detected FMDV genome at DPI 1 but only the NALHN and TetraCore assays detected FMDV genome at DPI 14. Viremia detected with any of the RRT-PCR assays also started at DPI 1 and was cleared within 4 – 5 days after detection (Figure 3).





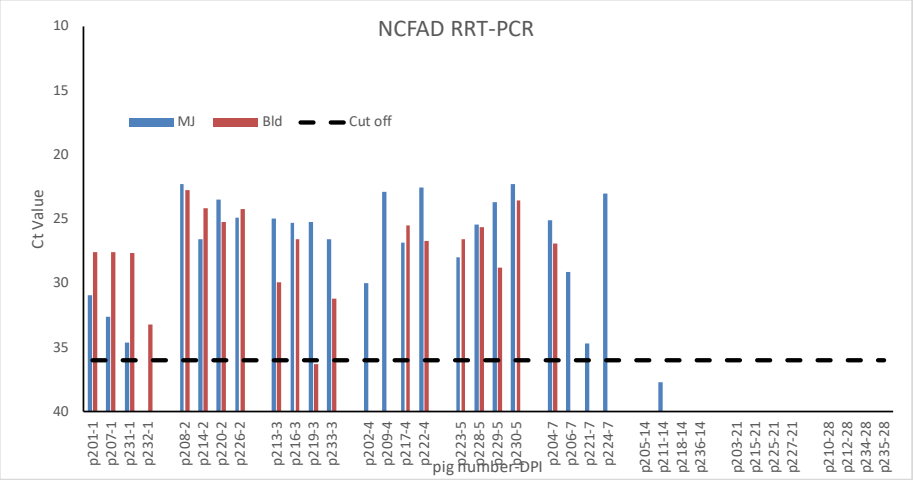


Figure 3: Detection of FMDV in meat juice and serum by 3 RRT-PCRs. Six groups of 6 pigs/group/room were used in this experiment. Three pigs per group were directly inoculated with 1000 $TCID_{50}$ of FMDV SAT2 Zim 5/81 by intradermal inoculation into the heel bulb. Starting at 1 day post infection (DPI) four pigs with the most severe clinical disease were euthanized and quadriceps muscle taken for meat juice collection after freeze-thawing. RNA was extracted from the meat juice and tested by each RRT-PCR. NAHLN= National Animal Health Laboratory Network, TetraCore = commercial supplier of FMDV RRT-PCR kits, NCFAD = National Centre for Foreign Animal Disease. DPI = days post infection

FMDV RNA was also detected in oral swabs starting at DPI 1 for most pigs and was still detectable at 14 - 28 DPI in oral swabs from some of the surviving pigs (Figure 4).

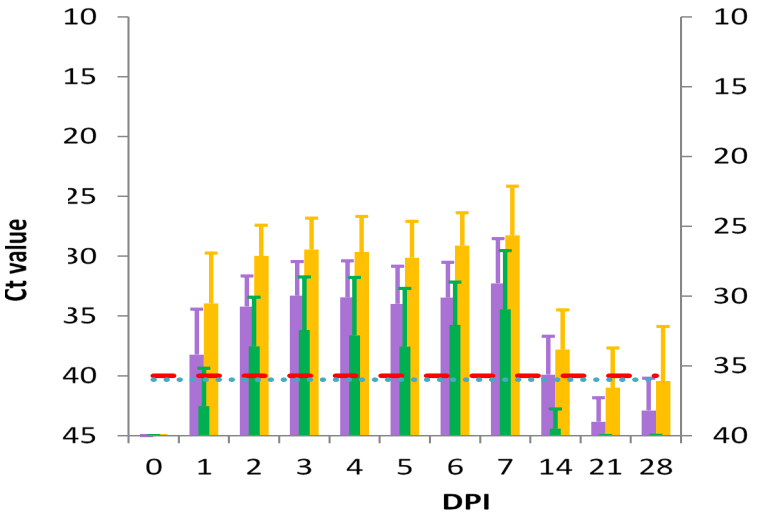


Figure 4: Crossing threshold (Ct) values of Foot-and-mouth disease virus (FMDV) genomic RNA detection in oral swabs from pigs infected with FMDV SAT2 Zim 5/81. RNA was extracted using MagMAX™-96 Viral RNA Isolation Kit, and tested with the ABI7500 Fast thermocycler using three different PCR reagent mixes. Threshold for the NCFAD RRT-PCR is 35.99, and 40 for the NAHLN and Tetracore RRT-PCRs. Samples with Ct values below the threshold are considered positive for FMDV

FMDV genome was detected at DPI 1 in oral fluids from all the groups (Table 3). Virus detection in oral fluids also mirrored detection in oral swabs.

	Day Post Infection (DPI)										
	0	1	2	3	4	5	6	7	14	21	28
Group 1	Und	Und	36.44	26.45	28.75	25.75	25.86				
	Und	35.06	30.79	23.92	26.19	23.38	23.81				
	Und	40.44	34.85	28.11	30.14	27.54	27.53				
Group 2	Und	Und	31.39	28.33	24.73	34.56	26.13	31.00	37.93	Und	Und
	Und	31.86	28.53	25.44	22.18	29.72	23.99	27.90	31.95	Und	40.89
	Und	36.50	32.31	29.54	26.42	33.91	27.58	31.92	35.10	Und	Und
Group 3	Und	Und	25.25	29.76	28.73	29.48	31.26	30.05		Und	
	Und	36.32	22.90	26.89	26.05	26.65	28.38	27.55		Und	
	Und	39.41	27.23	30.89	29.89	30.94	32.33	31.05		Und	
Group 4	Und	Und	27.78	28.88	32.25	35.72	29.45				
	Und	35.23	25.50	26.35	28.93	30.63	26.78				
	Und	40.27	29.21	29.95	32.44	34.43	30.93				
Group 5	Und	29.23	25.72	29.32	28.60	28.41	38.22	Und	Und	Und	
	Und	26.29	23.28	26.58	25.90	25.74	31.81	32.52	33.75	40.97	
	Und	30.69	27.67	30.64	29.92	29.85	34.60	35.89	36.52	40.27	
Group 6	Und	31.06	29.38	28.53	30.43	27.74	27.04		36.38	Und	Und
	Und	27.79	26.55	26.01	27.51	24.95	24.63		31.30	Und	Und
	Und	31.99	30.76	29.89	31.65	29.02	28.36		34.34	Und	44.15

Table 3: Crossing Threshold (Ct) RRT-PCR values of extracted oral fluids from the FMDV SAT2 Zim 5/81 experiment. RNA was extracted using MagMAX™-96 Viral RNA Isolation Kit, and tested with the ABI7500 Fast thermocycler using three different PCR reagent mixes. Threshold for the NCFAD RRT-PCR (green) is 35.99, and 40 for the NAHLN (purple) and TetraCore (orange) RRT-PCRs. Samples with Ct values below the threshold are considered positive for FMDV. Grey spaces indicate no sample was collected for that day. Und = undetermined

FMDV RNA was detected in the lymph nodes and tonsils of different animals starting at DPI 1. By DPI 2, all tested lymph nodes and tonsils were positive for FMDV RNA and stayed positive up to DPI 28 (Table 4). Overall, the NAHLN and TetraCore RRT-PCRs were more sensitive than the

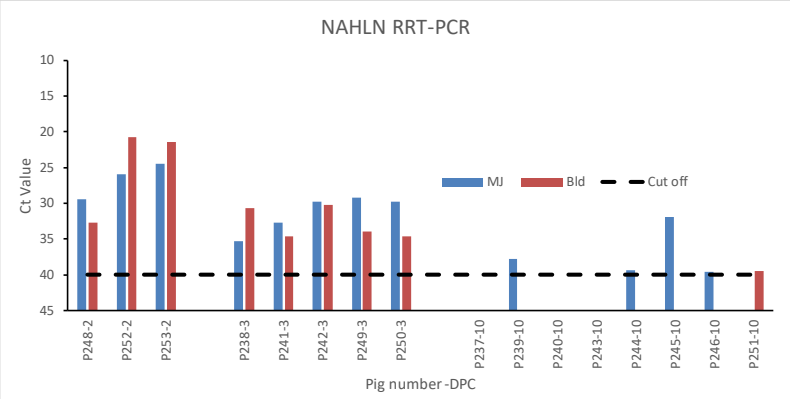
NCFAD RRT-PCR.

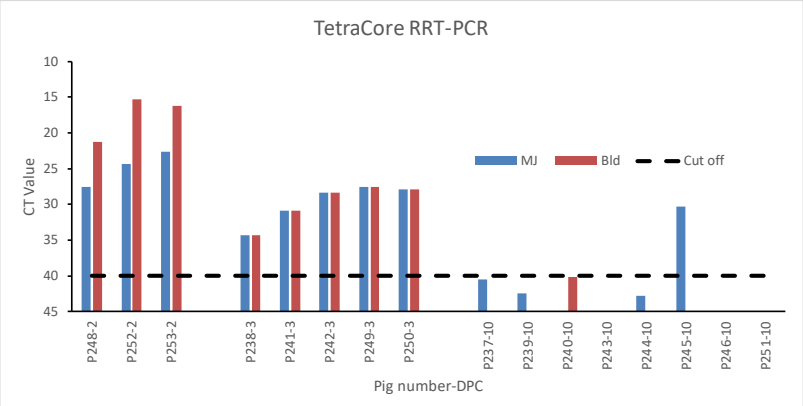
	NAHLN RRT-PCR				TetraCore RRT-PCR				NCFAD RRT-PCR			
Pig #-DPI	Submandibular	Prescapular	Popliteal	Tonsils	Submandibular	Prescapular	Popliteal	Tonsils	Submandibular	Prescapular	Popliteal	Tonsils
201-1	Und	Und	37.33	37.59	37.90	Und	37.60	35.97	Und	Und	39.27	35.51
207-1	Und	Und	Und	40.25	Und	Und	Und	36.41	Und	Und	Und	38.55
231-1	Und	38.98	Und	38.29	Und	Und	Und	33.84	Und	39.97	Und	Und
232-1	Und	Und	Und	37.16	Und	Und	Und	42.87	Und	Und	Und	Und
208-2	38.14	30.21	31.26	33.43	35.84	27.89	27.71	29.72	38.16	26.45	26.83	30.03
214-2	39.93	33.13	35.23	32.25	33.03	29.49	31.45	28.54	37.16	29.34	30.20	28.86
220-2	34.30	30.58	33.75	35.29	29.18	27.26	31.65	30.96	30.32	26.56	29.45	32.89
226-2	36.46	29.36	32.42	34.92	32.72	24.81	28.54	32.32	33.95	24.97	28.38	31.67
213-3	37.87	30.15	32.95	28.83	32.76	26.68	28.39	25.24	35.52	26.21	28.55	25.61
216-3	32.98	33.43	39.84	37.38	28.09	28.96	38.36	34.18	28.18	30.03	Und	34.83
219-3	Und	29.85	38.91	33.58	36.34	25.54	33.62	29.84	38.67	26.26	34.80	30.16
233-3	Und	30.11	35.10	36.34	35.07	26.14	32.47	31.78	38.16	26.29	32.47	33.21
202-4	Und	39.70	29.92	30.07	34.67	33.54	23.27	24.99	36.28	Und	25.57	26.29
209-4	36.24	31.02	31.24	29.43	29.40	23.23	23.65	26.27	32.36	26.69	27.02	25.25
217-4	37.99	32.35	28.98	34.31	32.00	24.52	23.32	31.28	37.29	27.34	24.03	30.16
222-4	35.68	30.74	35.92	32.50	30.61	23.53	29.87	28.09	31.72	26.33	33.07	28.59
223-5	39.87	34.75	29.90	36.49	35.18	28.21	23.03	31.97	Und	31.14	25.26	33.67
228-5	35.41	31.92	30.07	40.04	29.43	24.36	22.70	34.96	30.85	27.18	25.44	Und
229-5	37.49	31.92	31.23	33.97	33.83	25.28	23.89	30.10	36.90	27.18	25.95	30.01
230-5	31.76	33.29	29.54	29.67	28.93	26.63	22.16	25.05	28.05	28.55	24.47	25.96
204-7	31.70	25.57	27.71	30.49	28.16	20.48	21.21	25.82	27.48	21.08	22.64	26.15
206-7	28.58	29.43	29.84	26.65	25.44	25.85	26.17	25.01	24.52	25.23	24.93	23.74
221-7	Und	Und	Und	39.26	38.09	37.42	Und	35.77	Und	37.82	Und	35.72
224-7	37.93	36.59	Und	33.54	31.05	32.92	38.35	29.49	33.00	32.66	Und	28.80
205-14	Und	Und	39.58	37.59	41.30	40.13	34.55	33.44	Und	Und	35.74	33.11
211-14	Und	40.49	35.55	37.74	38.34	38.26	30.21	33.36	Und	Und	31.01	34.66
218-14	36.13	32.42	Und	36.16	31.38	27.47	Und	32.75	30.85	27.77	Und	33.53
236-14	36.29	38.74	34.80	37.08	30.77	33.99	29.35	32.46	31.09	37.14	29.84	34.74
203-21	Und	35.79	Und	39.51	35.97	32.50	Und	38.95	39.73	30.90	Und	Und
215-21	Und	34.74	Und	37.89	41.33	31.78	38.01	32.05	Und	29.90	Und	32.36
225-21	Und	Und	36.49	Und	Und	Und	33.37	40.37	Und	Und	31.77	Und
227-21	Und	35.11	Und	37.81	Und	31.32	38.77	32.90	Und	29.82	Und	33.16
210-28	Und	Und	Und	38.27	Und	Und	Und	35.96	Und	Und	Und	34.81
212-28	Und	37.84	38.88	Und	Und	36.45	35.03	36.56	Und	35.70	37.82	Und
234-28	38.95	Und	40.31	36.89	35.53	Und	34.44	33.31	36.95	Und	37.81	32.07
235-28	Und	Und	36.49	38.56	Und	40.69	33.15	36.02	Und	Und	31.99	36.76

Table 4: Crossing Threshold (Ct) RRT-PCR values of various extracted tissue suspensions (submandibular lymph nodes, prescapular lmph nodes, popliteal lymph nodes, and tonsils) from the FMDV SAT2 Zim 5/81 experiment. 10% tissue suspensions were prepared in PBS and clarified by centrifugation. RNA was extracted from tissue suspensions using MagMAX™-96 Viral RNA Isolation Kit, and tested with the ABI7500 Fast thermocycler using three different PCR reagent mixes. Threshold for the NCFAD RRT-PCR (green) is 35.99, and 40 for the NAHLN (purple) and TetraCore (orange) RRT-PCRs. Samples with Ct values below the threshold are considered positive for FMDV.

FMDV O1 Manisa experiment

In FMDV O1 Manisa experiment, FMDV genome was detected in MJ at days post challenge (DPC) 2 and 3 (Figure 5). The NAHLN and TetraCore RRT-PCRs detected FMDV RNA in more MJ samples at DPC 10 than the NCFAD RRT-PCR. Viremia was also detected at DPC 2 and 3 (Figure 5).





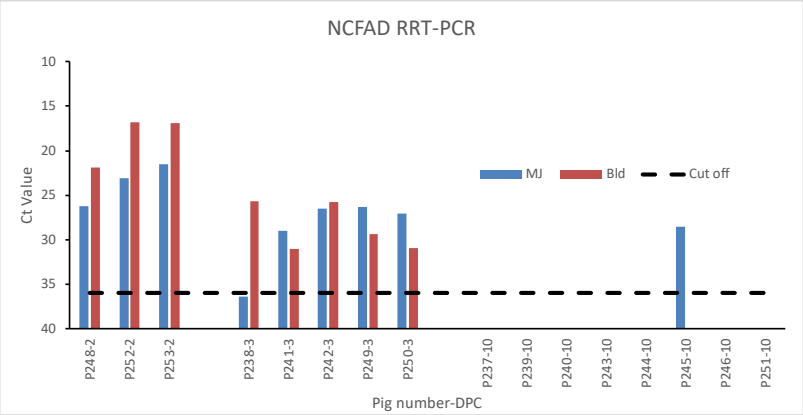


Figure 5: Fifteen pigs were vaccinated with a commercial BEI-inactivated FMD O1 Manisa vaccine and all 15 pigs plus 2 unvaccinated controls were challenged after 28 days. Meat juice was collected from the pigs showing clinical signs (lesions) and all surviving pigs euthanized at 10 days post-challenge for meat juice collection. RNA was extracted from the meat juice and tested by each RRT-PCR. NAHLN= National Animal Health Laboratory Network, TETRACORE = commercial supplier of FMDV RRT-PCR kits, NCFAD = National Centre for Foreign Animal Disease. DPC = days post challenge

FMDV ASIA1 Shamir experiment

In FMDV ASIA1 Shamir experiment, the sampling schedule was modified to every other day starting on DPI 2 except when an animal reached the humane end point. Consequently, 24 animals were used. However, 21 animals either died or reached humane end points and were euthanized in the acute phase. At DPI 14, the remaining 3 animals were euthanized. FMDV genome was detected in MJ at DPI 2 to as late as DPI 14, depending on the assay used (Figure 6). The TetraCore RRT-PCR was

more sensitive than the NAHLN and NCFAD RRT-PCRs (Figure 6). Specifically, only the TetraCore assay detected positive levels of FMDV genomes at DPI 14. Viremia detected with any of the RRT-PCR assays also started at DPI 2 and was cleared within 4 – 5 days after detection Figure 6).

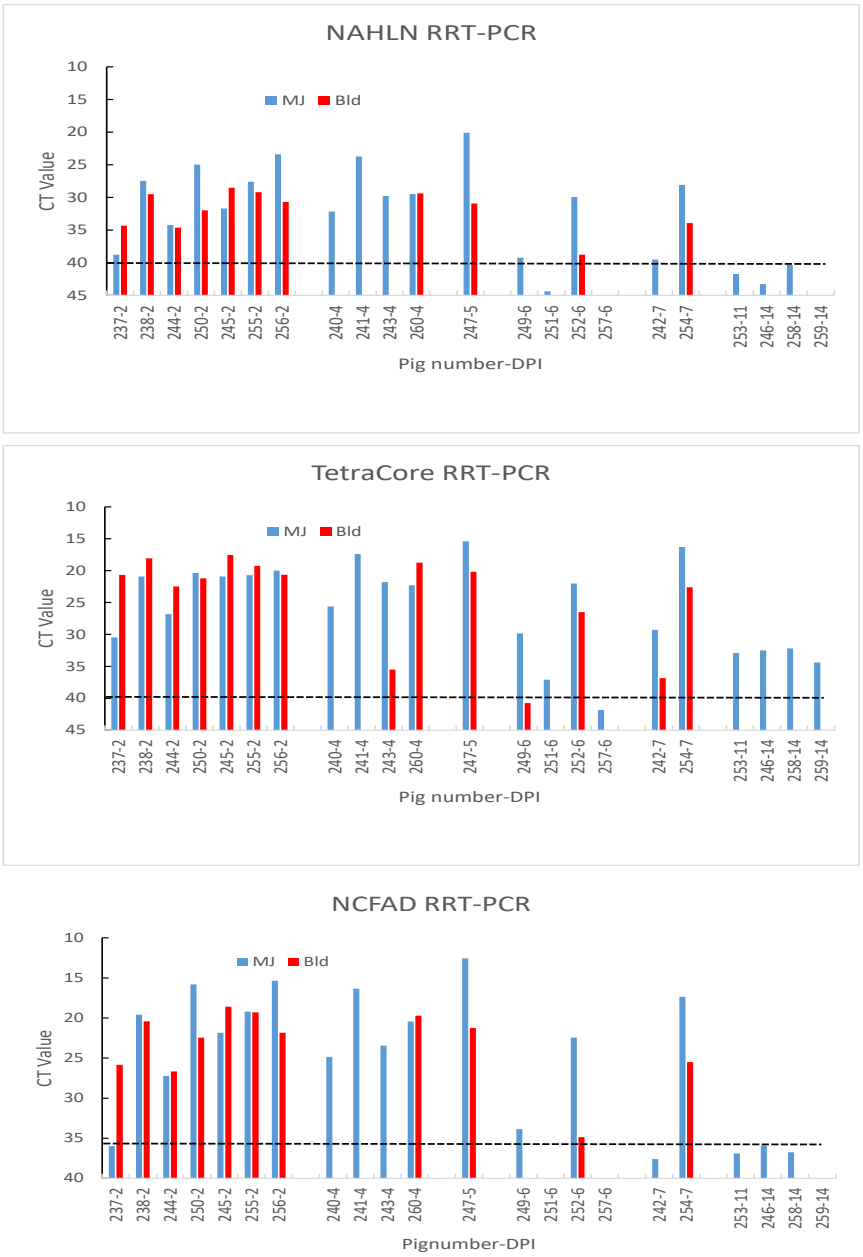


Figure 6: Detection of FMDV in meat juice and serum by 3 RRT-PCRs. Four groups of 6 pigs/group/room were used in this experiment. Three pigs per group were directly inoculated with 10,000 $TCID_{50}$ of FMDV ASIA1 Shamir by intradermal inoculation into the heel bulb. Starting at 2 days post infection (DPI) pigs with the most severe clinical disease were euthanized and quadriceps muscle taken for meat juice collection after freeze-thawing. RNA was extracted from the meat juice and tested by each RRT-PCR. NAHLN= National Animal Health Laboratory Network, TetraCore = commercial supplier of FMDV RRT-PCR kits, NCFAD = National Centre for Foreign Animal Disease. DPI = days post infection

FMDV RNA was also detected in oral swabs by the TetraCore assay starting at DPI 2 and was still detectable at DPI 14 (Figure 7). FMDV genome detection with the NCFAD and NAHLN assays was more limited (DPI 2 -6).

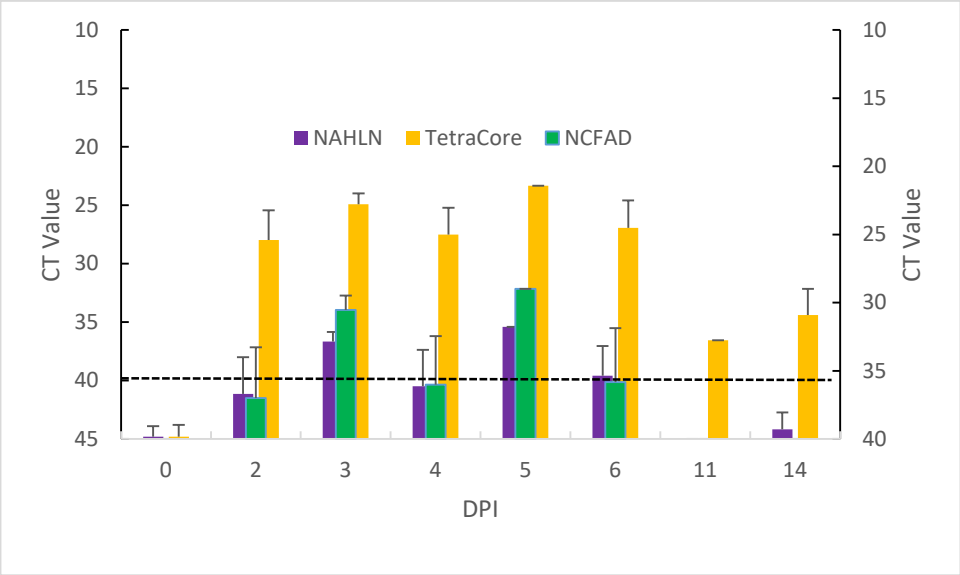


Figure 7: Crossing threshold (Ct) values of foot-and-mouth disease virus (FMDV) genomic RNA detection in oral swabs from pigs infected with FMDV ASIA1 Shamir. RNA was extracted using MagMAX™-96 Viral RNA Isolation Kit, and tested with the ABI7500 Fast thermocycler using three different PCR reagent mixes. Threshold for the NCFAD RRT-PCR is 35.99, and 40 for the NAHLN and Tetracore RRT-PCRs. Samples with Ct values below the threshold are considered positive for FMDV

FMDV genome was detected with all 3 assays at DPI 2 in oral fluids from all the groups (Table 5). Unlike oral swabs, none of the assays detected FMDV RNA at DPI 14.

	Day Post Infection				
	0	2	3	4	14
	Und	31.77	29.54		
Group 1	Und	37.52	34.22		
	Und	27.26	23.44		
	Und	29.12	Und	38.21	Und
Group 2	Und	34.58	Und	39.05	Und
	Und	24.54	33.27	27.69	Und
	Und	31.07	28.56	32.80	
Group 3	39.49	35.80	34.75	36.84	
	39.25	25.09	24.60	26.64	
	Und	28.66	25.48		
Group 4	Und	35.49	31.97		
	Und	25.77	27.59		

Table 5: Crossing Threshold (Ct) RRT-PCR values for FMDV RNA in oral fluids from the FMDV ASIA1 Shamir experiment. RNA was extracted using MagMAX™-96 Viral RNA Isolation Kit, and tested with the ABI7500 Fast thermocycler using three different PCR reagent mixes. Threshold for the NCFAD RRT-PCR (green) is 35.99, and 40 for the NAHLN (purple) and TetraCore (orange) RRT-PCRs. Samples with Ct values below the threshold are considered positive for FMDV. Grey spaces indicate no sample was collected for that day. Und = undetermined

FMDV RNA was detected in all tissues (submandibular, prescapular and popliteal lymph nodes, tonsil and heart muscle) from DPI 2 – 14 when using the TetraCore assay. On the contrary, only 16.5% and 27% of tissues were positive with the NCFAD and NAHLN assays respectively (Table 6).

			NAHLN RRT-PCR					Tetracore RRT-PCR				NCFAD RRT-PCR			
Pig #-DPI	Submandibular	Prescapula	Popliteal	Tonsils	Heart	Submandibular	Prescapular	Popliteal	Tonsils	Heart	bmandibu	rescapula	Popliteal	Tonsils	Heart
237-2	Und	44.27	44.63	43.15		35.46	34.06	30.69	31.89		Und	Und	Und	Und	
238-2	Und	38.57	44.50	35.05		30.48	29.02	29.31	28.00		Und	34.09	Und	30.57	
244-2	Und	37.73	38.49	40.92		34.29	24.44	25.99	30.96		Und	34.50	37.06	Und	
250-2	Und	40.35	Und	Und		30.12	26.75	32.51	33.00		Und	Und	Und	Und	
239-3	40.54	42.49		44.07	42.68	32.20	29.48		31.96	30.17	Und	Und		Und	Und
245-3	Und	Und	44.74	Und	39.95	34.06	32.60	32.96	32.32	28.50	Und	Und	Und	Und	36.75
255-3	Und	43.22	39.70	40.94	40.05	30.50	29.71	28.75	28.88	32.43	Und	Und	Und	39.36	Und
256-3	Und	39.98	Und	37.14	37.16	30.48	30.91	28.35	26.71	26.02	Und	Und	Und	33.31	31.58
240-4	Und	Und	41.46	Und	44.23	32.94	32.99	33.49	32.03	31.92	Und	Und	Und	Und	Und
241-4	Und	Und	40.26	43.91	37.03	33.21	33.49	31.20	31.97	25.16	Und	Und	Und	Und	30.92
243-4	Und	Und	Und	40.96		31.07	31.28	29.97	28.67		Und	Und	Und	37.43	
260-4	Und	42.08	40.27	41.30		30.81	27.31	28.00	28.92		Und	Und	Und	Und	
247-5	40.04	41.98		39.12	41.57	26.42	26.67		29.16	29.14	Und	39.55		38.01	37.01
248-6	Und	42.25	42.51	43.81	42.68	32.59	29.99	27.91	31.17	31.58	Und	Und	39.28	Und	Und
249-6	39.86	42.30	Und	38.81		26.43	29.13	28.98	26.11		Und	Und	Und	36.41	
251-6	43.10	41.40	39.62	42.56		28.54	30.36	25.57	27.76		Und	Und	Und	Und	
252-6	41.76	37.67	40.96	39.00		26.62	24.10	24.00	28.82		Und	34.95	38.50	Und	
257-6	31.98	37.40	37.65	37.48		25.58	27.66	25.34	26.82		27.82	34.01	36.53	33.88	
242-7	41.73	41.78	41.34	37.41		29.82	26.85	26.00	26.59		Und	39.69	38.74	35.13	
254-7	37.49	41.34	40.34	35.28	39.44	27.52	26.31	23.84	25.85	28.40	36.07	determin	35.89	31.20	35.04
253-11	Und	43.33	42.92	Und		32.06	29.87	25.30	33.37		Und	37.72	Und	Und	
246-14	Und	39.24	Und	42.95		31.52	26.69	31.11	28.13		Und	36.03	Und	39.93	
258-14	42.96	Und	39.18	37.36		30.02	31.45	26.26	26.95		Und	Und	33.73	32.88	
259-14	Und	44.77	Und	37.86		34.63	30.49	31.30	28.57		Und	Und	Und	34.04	

Table 6: Crossing Threshold (Ct) RRT-PCR values FMDV RNA in tissue suspensions (submandibular lymph nodes, prescapular lymph nodes, popliteal lymph nodes, tonsils and heart) from the FMDV ASIA1 Shamir experiment. 10% tissue suspensions were prepared in PBS and clarified by centrifugation. RNA was extracted from tissue suspensions using MagMAX™-96 Viral RNA Isolation Kit, and tested with the ABI7500 Fast thermocycler using three different PCR reagent mixes. Threshold for the NCFAD RRT-PCR (green) is 35.99, and 40 for the NAHLN (purple) and TetraCore (orange) RRT-PCRs. Samples with Ct values below the threshold are considered positive for FMDV.

Detection of FMDV antigen in meat juice by lateral flow strip test

The data for serotypes A and SAT 2 antigen detection in meat juice has been published " Detection of Foot-and-Mouth Disease Virus in Swine Meat Juice. Yeo S, Yang M, Nyachoti M, Rauh R, Callahan JD, Nfon C. Pathogens. 2020 May 29;9(6):424. doi: 10.3390/pathogens9060424.Pathogens. 2020. PMID: 32485851

For the FMDV ASIA1 Shamir experiment, FMDV antigen was detectable at 2 - 6 DPI by lateral flow strip test (Figure 8). Twenty four MJ samples from naïve pigs were negative for FMDV ASIA1 Shamir antigen by this test (data not shown).

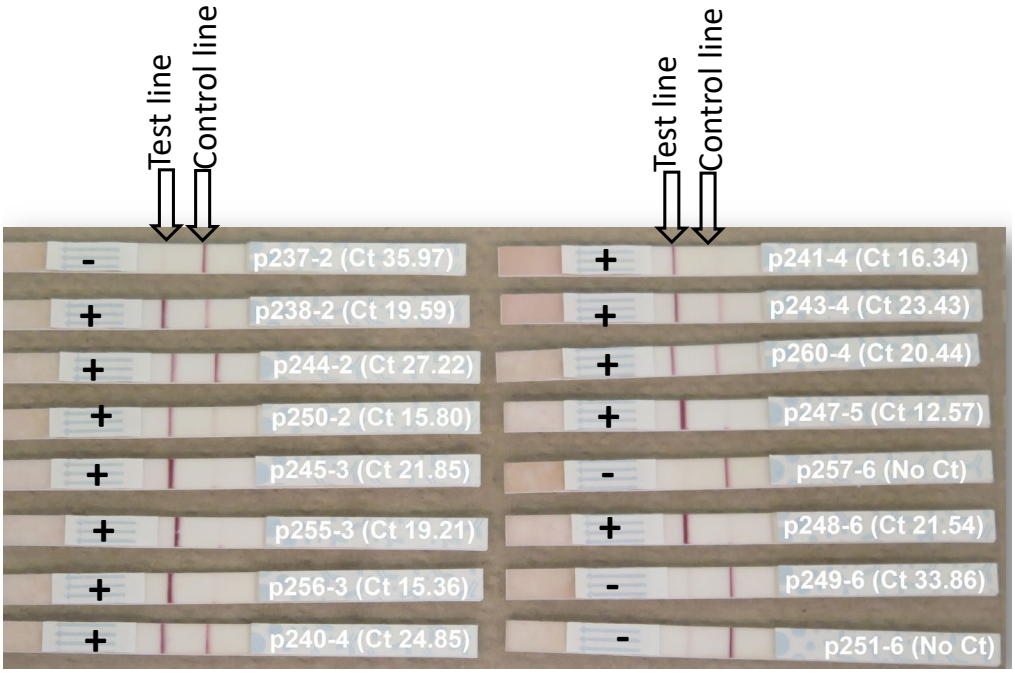


Figure 8: Detection of FMDV ASIA1 Shamir antigen in meat juice by lateral flow strip test. Four groups of 6 pigs/group/room were used in this experiment. Three pigs per group were directly inoculated with 10,000 $TCID_{50}$ of FMDV ASIA1 Shamir by intradermal inoculation into the heel bulb. Starting at 2 days post infection (DPI) pigs with the most severe clinical disease were euthanized and quadriceps muscle taken for meat juice collection after freeze-thawing. Meat juice was tested for FMDV serotype ASIA1 antigen by lateral flow strip test. + = positive for FMDV antigen, - = negative for FMDV antigen, DPI = days post infection

Detection of antibodies to FMDV in meat juice by ELISA

Meat juice was not suitable for detection of antibodies to non-structural proteins (NSP) using commercial and NCFAD FMD 3ABC competitive ELISAs (cELISAs). An alternate assay is being developed using available NCFAD reagents.

However, antibodies to FMDV structural proteins (SP) were detected in meat juice using in-house serotype-specific cELISAs. Positive levels of anti-FMDV SP antibodies were detected in MJ at DPI 12 and remained relatively high at DPI 21 for the A22 IRQ 24/64 experiment (Figure 9A). There was a correlation between anti-FMDV SP antibody detection in MJ and sera from corresponding animals (r2 = 0.77; P<0.0001).

For the SAT2 experiment, positive levels of anti-FMDV SP antibodies were detected in MJ samples starting at DPI 7 (Figure 9B). There was also a correlation between anti-FMDV SAT2 SP antibody detection in MJ and sera from corresponding animals (r2 = 0.84; P<0.0001).

For the ASIA1 experiment, anti-FMDV SP antibodies were detected in all samples at DPI 14 (Figure 9C). However 75% of sera from corresponding animals were positive at DPI 6 as opposed to zero for MJ at this time point. Nevertheless, there was a correlation between anti-FMDV ASIA1 SP antibody detection in MJ and sera from corresponding animals (r^2 = 0.64; P<0.0002).

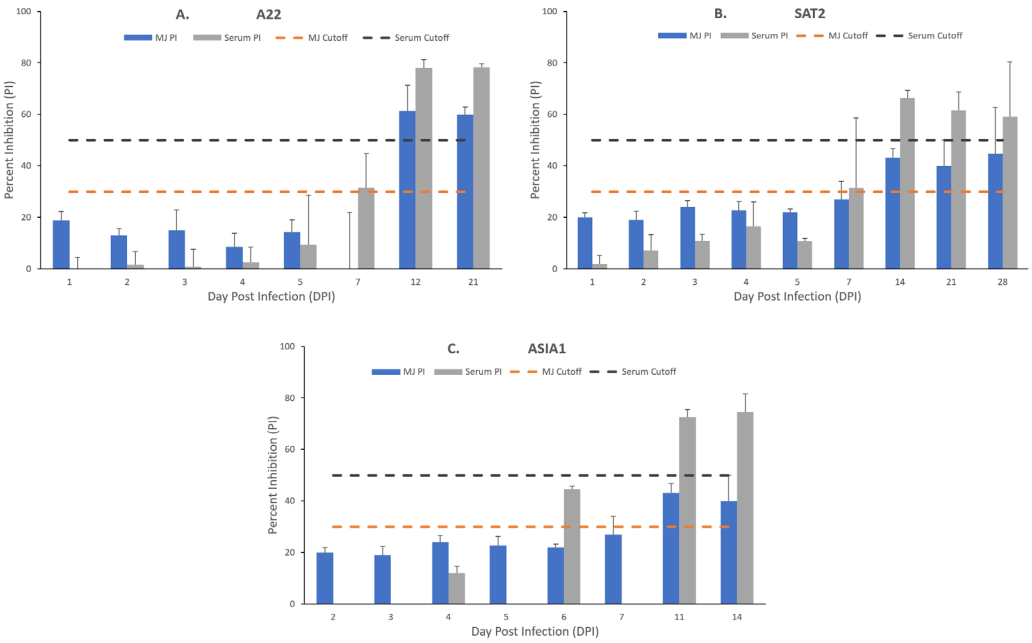


Figure 9. Detection of antibodies to FMDV structural proteins in meat juice and sera from FMDV-infected pigs. Percent Inhibition (PI) values show the level of antibodies to structural proteins of FMDV in meat juice (MJ) collected from the biceps femoris and serum from pigs experimentally infected with (A) A22 IRQ 24/64, (B) SAT2 ZIM 5/81, and (C) ASIA1 Shamir. The cutoff PI value for MJ is 30. The cutoff value for serum is 50.

For the FMDV O1 Manisa experiment, antibodies levels were below the cut off at DPC 2 and 3 but all samples were positive for antibodies to FMDV structural proteins at DPC 10 (Figure 10). The serum antibody response mirrored that of MJ. Note that animals euthanized at DPC 2 and 3 had responded poorly to the vaccine at 28 days post vaccination (DPC 0) while the animals that seroconverted to the vaccine survived to DPC 10, hence the high antibody levels in MJ and sera at DPC 10.

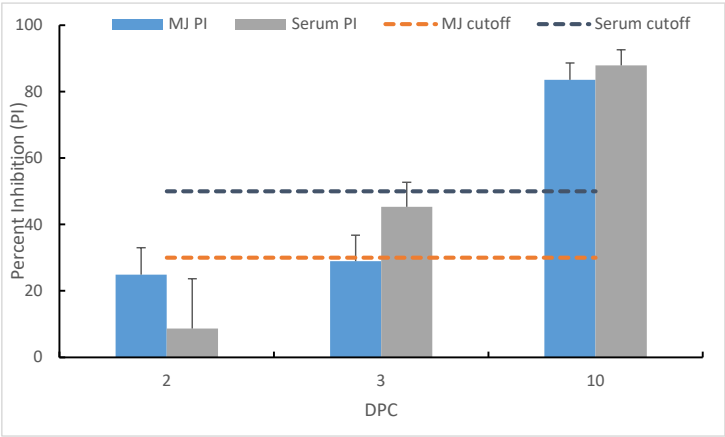


Figure 10: Detection of antibodies to FMDV O structural proteins in meat juice and serum by serotype specific competitive ELISA. Fifteen pigs were vaccinated with a commercial BEI-inactivated FMD O1 Manisa vaccine and all 15 pigs plus 2 unvaccinated controls were challenged after 28 days. Meat juice was collected from the pigs showing clinical signs (lesions) at 2 and 3 days post-challenge and all surviving pigs euthanized at 10 days post-challenge for meat juice collection. Meat juice was tested for antibodies to structural proteins by FMDV serotype O cELISA. PI = percent inhibition, DPC = days post challenge

Oral fluids have been collected from the animal experiments described above and tested by RRT-PCR. Some of these (FMDV serotypes A and SAT 2) were also used in a collaborative project with Iowa State University to validate a FMD 3ABC ELISA for swine oral fluids. More samples are available for sharing with qualified laboratories under appropriate agreements.

IX. Discussion:

Our data shows that FMDV can be detected in MJ primarily by RRT-PCR. In addition, FMDV antigen can be detected in MJ at specific time points using LFSTs. This study also compared 3 RRT-PCR assays for detection of FMDV in MJ and other sample types. The NALHN and TetraCore RRT-PCR assays were more sensitive than the NCFAD RRT-PCR. The differences were most obvious early post infection (DPI 1 – 2) before the onset of clinical signs and at the late time points when the animals were recovering from disease. This could be attributed to the fact that in the preclinical and the convalescent phases of the disease, the amount of nucleic acid in the samples is likely low, falling below the detection limit for the NCFAD assay. This therefore indicates better analytical sensitivities for the NALHN and TetraCore assays. Overall, the TetraCore and the NAHLN assays were comparable across all sample types. This is consistent with previous finding using the lyophilized forms of these assays (Howson et al, 2018).

Based on the NAHLN RRT-PCR assay, MJ and serum samples from the same pigs were positive for FMDV RNA starting from DPI 1 - 7. In the absence of viremia, MJ samples from DPI 12 – 21 were positive for FMDV genome. It is possible that the clearance of the virus from muscles happens at a slower rate than from blood, possibly because the virus is located within the myocytes or adipocytes and only released when cells are disrupted by freeze-thawing. The second possibility is that the virus could have originated from the regional lymph nodes and released into muscles through the lymphatic system. FMDV is known to persist in popliteal lymph nodes for more than 28 days post infection (Stenfeldt et al, 2016). We also showed that lymph nodes from infected pigs were positive for FMDV RNA at the end of the experiments at DPI 14, 21 and 28 for FMDV ASIA1, A and SAT2 respectively. Detection of FMDV antigen in MJ by LFSTs shows this test can be used at point of need to test illegally imported fresh or frozen meat.

We have also shown that antibodies to FMDV SP can be detected in MJ concurrent with detection in serum. Antibodies to FMDV SP begin circulating in serum of most infected animals at 5 - 7 DPI. Since MJ is a mixture of serum and other cellular exudates, presence of antibodies in serum should result in antibodies in MJ and this is supported by our data. Sera from FMDV-infected animals usually permit for diagnosis of ongoing infection through viral nucleic acid, and resolved infection through the detection of antibodies. Thus, as revealed by our data, MJ provides similar diagnostic opportunities as serum with the additional benefit that FMDV RNA is detected in MJ for a longer period than in serum. This allows for a longer overlapping period (DPI 6/7 - 21) during which both FMDV nucleic acids and antibodies to FMDV SP can be detected in the same MJ sample. FMDV detection in serum is usually short-lived, with viremia disappearing before DPI 7.

In conclusion, MJ is a useful sample for FMDV detection alongside traditional sample types and also in exceptional circumstances when the usual samples are no longer available. In addition, MJ is a suitable sample for the detection of antibodies to FMDV SP. Taken together, this sample type is valuable for the diagnosis of FMD during the acute and convalescent phases of the disease.

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