

final report

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qRT-PCR to detect FMD virus

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Executive Summary

Of all exotic diseases, foot and mouth disease (FMD) presents the greatest threat to Australia's livestock industries. An outbreak of FMD would have a severe impact through loss of both productivity and exports. This virus can be difficult to detect in sheep and there has been significant evidence of dispersal of FMD virus (FMDV) through the movement of undetected, infected sheep. Consequently, there is a need for a laboratory based method for rapid testing to support large scale surveillance. Recent research has shown that FMDV can be detected in oral fluids/oral swabs for lengthy periods using virus isolation/culture indicating that there is merit in using oral swabs for FMDV surveillance because of the ease of sample collection and capacity to detect virus for longer than from blood. Virus isolation is, however, labour intensive, expensive and relatively slow and amplifies infectious virus, requiring expensive containment laboratory facilities. In contrast, quantitative 'real time' reverse transcription polymerase chain reaction (qRT-PCR) technology is very sensitive and allows rapid detection of viral nucleic acid (RNA) without amplifying infectious virus. A combination of qRT-PCR assays to detect FMDV RNA and collection of oral swabs would provide an efficient option for large scale FMDV surveillance – either during an outbreak or during 'proof of freedom' testing. Since such an approach would be of considerable value in Australia, the McGarvie Smith Institute and NSW Department of Primary Industries, in partnership with MDC, provided funds to undertake such a study. A large collection of plasma and oral swab samples from experimentally infected sheep was available at the Central Veterinary Institute, Lelystad, The Netherlands and were offered for use in this research. These were invaluable because the time of infection and stages of infection were accurately defined and virus isolation, a 'gold standard against which to evaluate the high throughput qRT-PCR assay, had been completed on each sample. The management of the German Federal Reference Laboratories at the Friedrich Loeffler Institute (FLI), Greifswald agreed to host Australian scientists to undertake this study.

The objectives of this project were to:

- Confirm the capacity of qRT-PCR to detect FMDV in oral swabs from sheep;
- Define the onset and duration of detection of FMDV in oral fluid and plasma;
- Estimate the level of pooling of samples that might be achieved without compromising the capacity to detect FMDV in a flock

Approximately 3,000 samples from experimentally infected sheep were transferred from The Netherlands to FLI, Germany immediately prior to arrival of the Australian scientists. Laboratory reagents were supplied from Australia and equipment matching what is used at EMAI, Menangle for large scale emergency animal disease testing was available at FLI. The qRT-PCR assay detected FMDV RNA in almost all oral swabs collected in the first 7-10 days after infection and 65% of samples were still positive at 30 days post infection. In contrast virus was only detected in plasma for a short time. Virus culture was also less sensitive than the qRT-PCR when testing either oral swabs or plasma. The results of sample pooling studies showed that it was possible to detect a single positive sample in a pool of ten samples during the first week of infection and to detect one positive in a pool of at least 5 samples one month after animals had been exposed to FMDV.

The combination of testing of oral swabs by high throughput qRT-PCR adds a new dimension to the detection of FMDV in sheep and will facilitate surveillance in the event of a disease outbreak. As the virus levels in sheep are low compared to cattle and pigs, it is expected that this sampling strategy can also be readily applied to monitoring of these species.

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1 Background

Of all exotic diseases, foot and mouth disease (FMD) presents the greatest threat to Australia's livestock industries. FMD virus (FMDV) can infect cattle, sheep, goats, pigs and a number of other animal species. While an outbreak of FMD in Australia's susceptible livestock would have a severe impact from disease and loss of productivity, the economic impact through loss of export markets would be devastating, with long term consequences even after control of an outbreak and eradication of the virus. Infection of sheep with FMDV is frequently sub-clinical. When clinical signs are apparent, they may be mild and difficult to differentiate from other diseases. Consequently, there are situations where there has been significant dispersal of FMDV following the movements of sheep. The possibility that sheep flocks could provide a reservoir of virus is a concern for countries that are free of FMDV. Consequently, there is a need for a laboratory based method for rapid testing to support large scale surveillance.

The observation that FMDV can be detected in oral fluids/oral swabs for lengthy periods using virus isolation/culture, and for much longer than can be detected in serum, suggests that there is merit in using oral swabs for FMDV surveillance. Further, virus isolation is labour intensive, expensive and relatively slow, as well as amplifying infectious virus, requiring high security laboratory facilities. In contrast, real time reverse transcription polymerase chain reaction (qRT-PCR) technology is very sensitive and allows rapid detection of viral nucleic acid without amplifying infectious virus. When combined with real time PCR assays to detect FMDV RNA, the collection of oral swabs would provide an efficient option for sample collection and testing during FMDV surveillance – either during an outbreak or during 'proof of freedom' testing. As such an approach would be of considerable value in Australia, the McGarvie Smith Institute and NSW Department of Primary Industries, in partnership with MDC, provided funds to undertake such a study.

A large collection of plasma and oral swab samples from experimentally infected sheep was available at the Central Veterinary Institute, Lelystad, The Netherlands. Unlike field collected specimens, samples from experimentally infected animals are preferred for the evaluation of the capacity of a qRT-PCR to detect FMDV because the time of infection and stages of infection are accurately defined. There is also a need to have a 'gold standard' against which to evaluate an assay. The samples that were offered by scientists from Lelystad met these requirements because all had been collected from experimentally infected animals and each sample had been tested by virus isolation. These samples were made available for this research.

2 Projective objectives

The objectives of this project were to:

- 2.1 Confirm the capacity of quantitative real-time PCR (qRT-PCR) to detect FMDV RNA in oral fluids and oral swabs from sheep;
- 2.2 Define the onset and duration of detection of FMDV in oral fluids, swabs and serum;
- 2.3 Estimate the level of pooling of samples that may be achieved without compromising the capacity to detect FMDV in a flock.

3 Methodology

3.1 Project location

As Australian laboratories are not permitted to handle “live” FMDV, this project was undertaken offshore. After exploring several options to test the samples in an overseas laboratories, collaborators at the Friedrich Loeffler Institute (FLI) in Germany agreed that they would be willing to receive the samples and to host Australian scientists to undertake this testing. While this initiative complements a long-standing international collaboration between EMAI and FLI, this was also the most practical location for this research due to its proximity to Lelystad. Rather than dealing with the challenges of long distance air-freight to another laboratory, short distance road transport is generally much less problematic.

3.2 Sample selection and transport

The research group at Lelystad have conducted experimental evaluations of FMDV vaccines in both sheep and cattle and have also investigated inter-species transmission between both vaccinated and unvaccinated animals. However, it is highly unlikely that sheep would be vaccinated in an FMD control program in Australia. As the aim was to assess the capacity of qRT-PCR to detect infected unvaccinated animals, the emphasis was placed on selection of samples from unvaccinated, challenged sheep. Nevertheless, due to the logistical difficulties associated with removal of specific individual samples from large batches of samples clustered in cryogenic storage systems, some samples from vaccinated sheep were also shipped. In order to provide a ‘benchmark’ plasma samples were also selected from some groups of animals that had been proven to be viraemic by virus isolation. Ultimately, samples of serum and oral swabs were shipped from sheep in 4 different transmission trials. These sheep had been sampled daily for 21-28 days from infection with FMDV. Approximately 2000 samples were available for testing. The samples were transferred from CVI Lelystad to FLI, Reims Island using a dedicated dangerous goods courier service.

3.3 Laboratory methods

The concept underlying this project was to be able to apply high-throughput qRT-PCR methods that are in routine use at EMAI and have been shown to be highly effective during the laboratory response to outbreaks of avian and equine influenza. Critical components included a semi-automated nucleic acid purification system and real time PCR technology that, during a disease outbreak, allows several thousand samples to be processed per day with 3-4 laboratory technicians. Specific details are as follows:

3.3.1 Sample processing

Due to very strict German Federal regulations related to the handling of infectious FMDV in an FMDV-free country, the FMDV laboratory at FLI has rigid protocols that cannot be modified without regulatory approval. These are designed to ensure that FMDV is fully inactivated before being transferred from the high security BSL3 (Ag) FMDV facility to a secure BSL2 laboratory where the RNA extraction takes place. Consequently, there had to be some modification of reagents that would be routinely used in Australia. In particular, an alternative sample lysis buffer (AL) is used at FLI during the initial processing of samples. Further, to ensure that no infectious virus leaves the FMDV laboratory, samples are also heated at 70°C for 20 minutes. Prior to the Australian scientists arriving at FLI, trials were undertaken to determine the compatibility of the AL buffer with MagMax sample lysis buffer and to evaluate ‘down-stream’ bead washing protocols that are used at FLI. These studies largely involved processing of porcine enterovirus that was used as proxy for FMDV. The effects of the AL buffer and heating on an exogenous internal control that is used at EMAI to

monitor the efficiency of the extraction and to check for inhibition of the nucleic acid amplification assay were also evaluated.

3.3.2 RNA extraction

Since the aim of this project is to simulate systems used in Australia, the Magmax viral RNA extraction kit was used for total nucleic acid extraction and run on a Biosprint magnetic particle handling system that is the same as the Kingfisher 96 (KF96) platform used at EMAI. The volumes of sample, lysis buffer and elution buffer and the wash times were comparable to those used at EMAI. A commercial RNA preparation (Xeno RNA) was used as the exogenous internal control and was included in the sample lysis buffer to monitor efficiency of RNA extraction and detect potential PCR inhibitors.

3.3.3 qRT-PCR

The qRT-PCR was based on the standard OIE approved '3D' assay using AgPath-RT mastermix. Assay cycling parameters were the standard conditions specified for AgPath mastermix. The primers and probe for the exogenous internal control (Xeno RNA) were also included and run as a duplex assay with the FMDV reagents.

3.3.4 Sample pooling

The main objective of sample pooling is to reduce the number of samples to be tested. However, pool size must be practical and not significantly compromise analytical sensitivity. The time and cost for preparing pools must be offset against the cost of testing of individual samples. For surveillance purposes, it is unlikely that extremely large numbers of samples would be collected from a single 'epidemiological unit' (eg separate management groups on a farm). Based on epidemiological advice, it is unlikely that batches of more than 30-50 samples would be collected. Hence very large pools are not envisaged and, consequently, pools of 3, 5, or 10 samples could be practical. As the aim was to determine the analytical sensitivity of different pool sizes, it was considered that the greatest information would be derived from pools created by the inclusion of single positive samples. (There is little value in pooling multiple positive samples apart from demonstrating the extent to which Ct values might vary). An important consideration was to understand whether testing of samples pooled at different stages of infection had an impact on analytical sensitivity. RNA levels are likely to be different soon after infection compared to the latest time at which RNA may be detected. To investigate these issues, pools of samples were prepared at Day 2 and Day 28 of infection. Although in practice individual samples would be pooled, a practical consideration for this project was whether sample pools should be prepared for RNA extraction or whether RNA that had already been extracted should be pooled. The latter approach was selected as it would not only save time but would also reduce the use of expensive reagents used for RNA extraction.

3.3.5 Sourcing/supply of reagents

All critical reagents that were needed for RNA extraction and for the qRT-PCR were sourced through Australian distributors to match reagents and other consumables that are routinely used at EMAI. Arrangements were made for these materials to be shipped to FLI prior to arrival of the Australian scientists.

4 Results

4.1 Samples transport and handling

4.1.1 Sample transfer

The samples arrived safely at FLI Reims Island a few days before the arrival of the Australian scientists and were stored at -80°C until tested. The scientists at CVI Lelystad provided spreadsheets documenting sample identification and linkages back to previous studies and the associated results. Samples were obtained from 3 studies in which sheep had been infected with FMDV serotype “O Manisa” or FMDV serotype “Asia 1”. The shipment included approximately 1650 oral swabs and 725 plasma samples.

4.1.2 Sample inactivation

Samples were stored in micro-storage tubes in an 8x12 array with a volume of approximately 800uL per tube. Using a biological safety cabinet in the BSL3 (Ag) laboratory, an appropriate volume of sample was added to AL sample lysis buffer in deep well storage plates, heat sealed with an aluminium foil sheet and then heated at 80°C for 20 minutes. The deep well plates were transferred out of the laboratory through a dunk tank and taken to the BSL2 laboratory for RNA extraction. The Xeno RNA was not subjected to heating but was included by addition with other reagents prior to extraction but after the heating and removal from the BSL3 laboratory.

4.2 qRT-PCR – Individual samples

Conventional methods of FMDV diagnosis have included virus isolation on both blood and affected tissues (either swabs from the lesions or homogenised tissue). Consequently plasma was available from the original virus transmission studies, along with the oral swabs. Although the published virus isolation studies suggested that virus could be detected in plasma for a shorter time than in oral swabs, a selection of plasma samples was tested to provide baseline data and to link with recognised diagnostic and surveillance methods.

4.2.1 Plasma

Plasma samples were tested from 20 sheep infected with FMDV Asia 1. Samples were available for Day 1 to Day 12 post infection. FMDV RNA was detected from Day 2 through to Day 9 with peak detections on Days 3-5 and Days 8 and 9 (Figure 1). On these days 60-75% of animals gave positive results.

4.2.2 Oral swabs

Oral swabs were available from 20 sheep infected with FMDV Asia 1 for a 31 day period. FMDV RNA was detected by qRT-PCR throughout this period, and from more than 50% of animals on all but 2 days (45% on Day 11 and 30% on Day 30) (Figure 2).

Similar results were obtained for a group of 12 unvaccinated sheep that were infected with FMDV serotype O Manisa. While these sheep were only sampled for 11 days after inoculation, infection was detected in a very high proportion of sheep. Virus was detected by qRT-PCR in 11 of the 12 sheep on 7 of the 11 days on which they were sampled (Figure 3).

4.2.3 Comparison with virus isolation

The results of these studies consistently demonstrate the superior sensitivity of real time PCR compared to virus isolation in cell culture. Whether detecting virus in plasma or oral swabs, there were fewer positive samples detected by virus isolation than by qRT-PCR (Figures 1-3). From about Day 7 after inoculation, the difference in favour of qRT-PCR becomes even more marked, perhaps due to the inhibitory effects of antibodies on virus replication. If sample volume was to be taken into consideration, these differences would be even greater because real time PCR analyses 50-100 fold less sample than the minimum that is used for virus isolation.

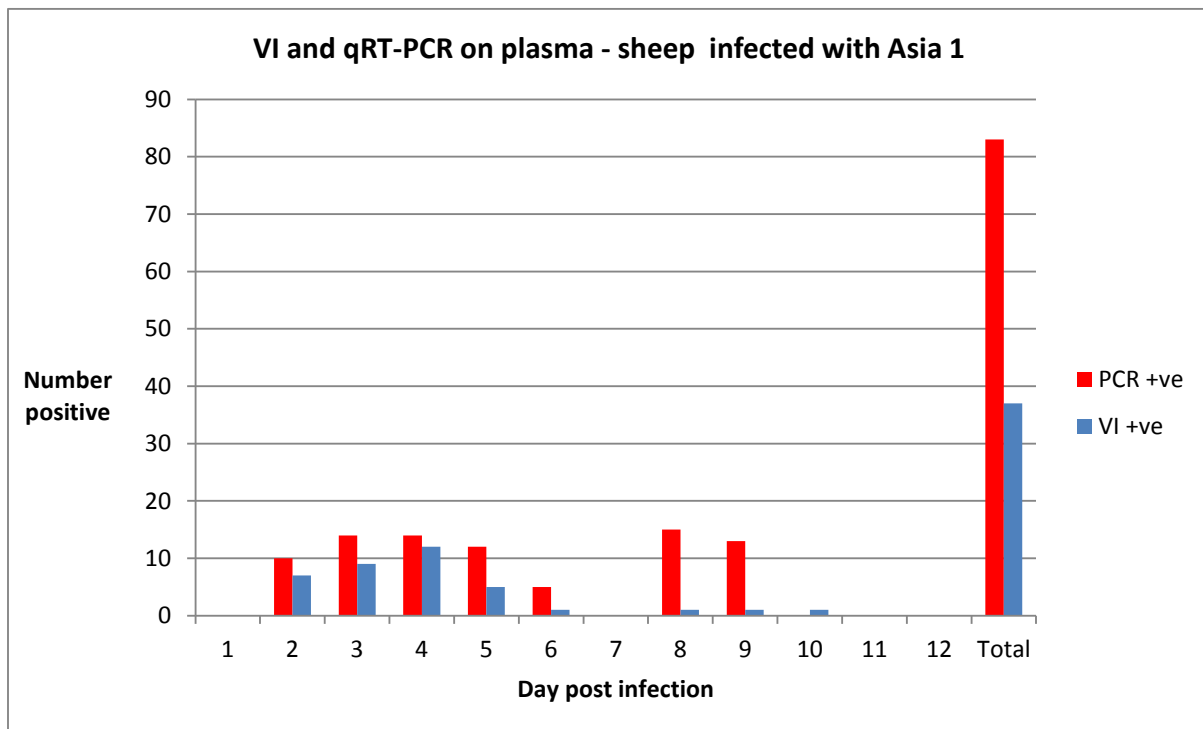


Figure 1. Detection of FMDV in plasma by qRT-PCR and virus isolation.

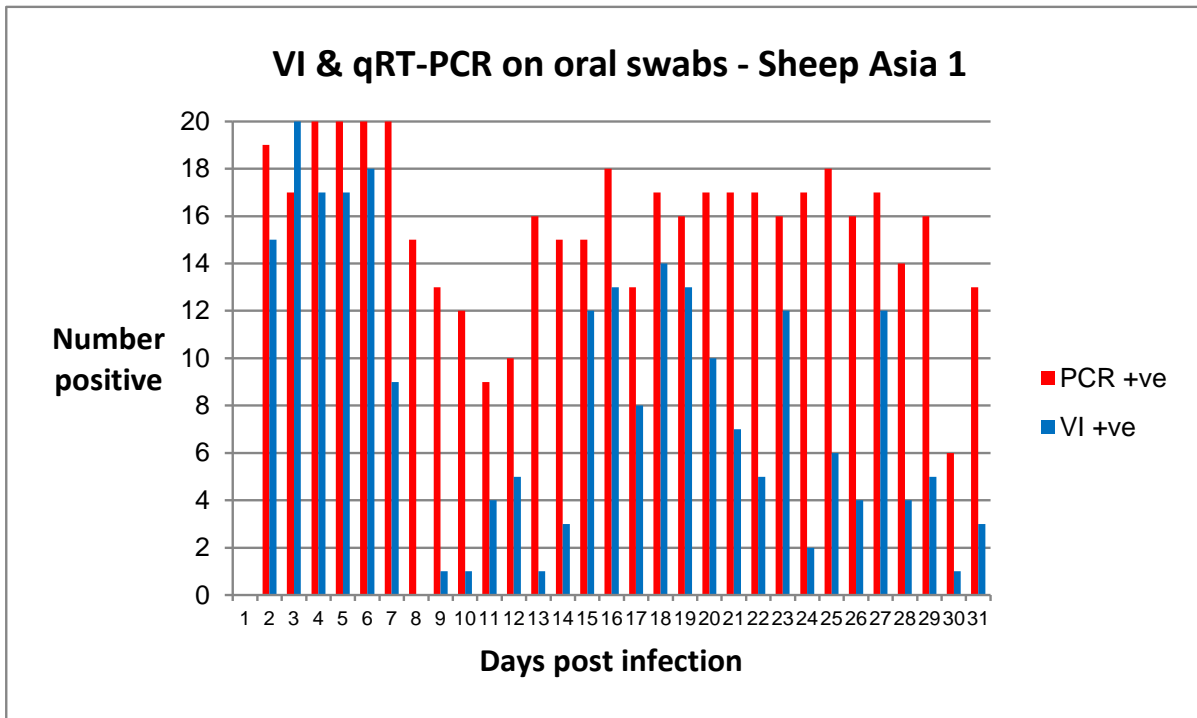


Figure 2: Detection of FMDV in oral swabs of sheep infected with FMDV serotype Asia 1.

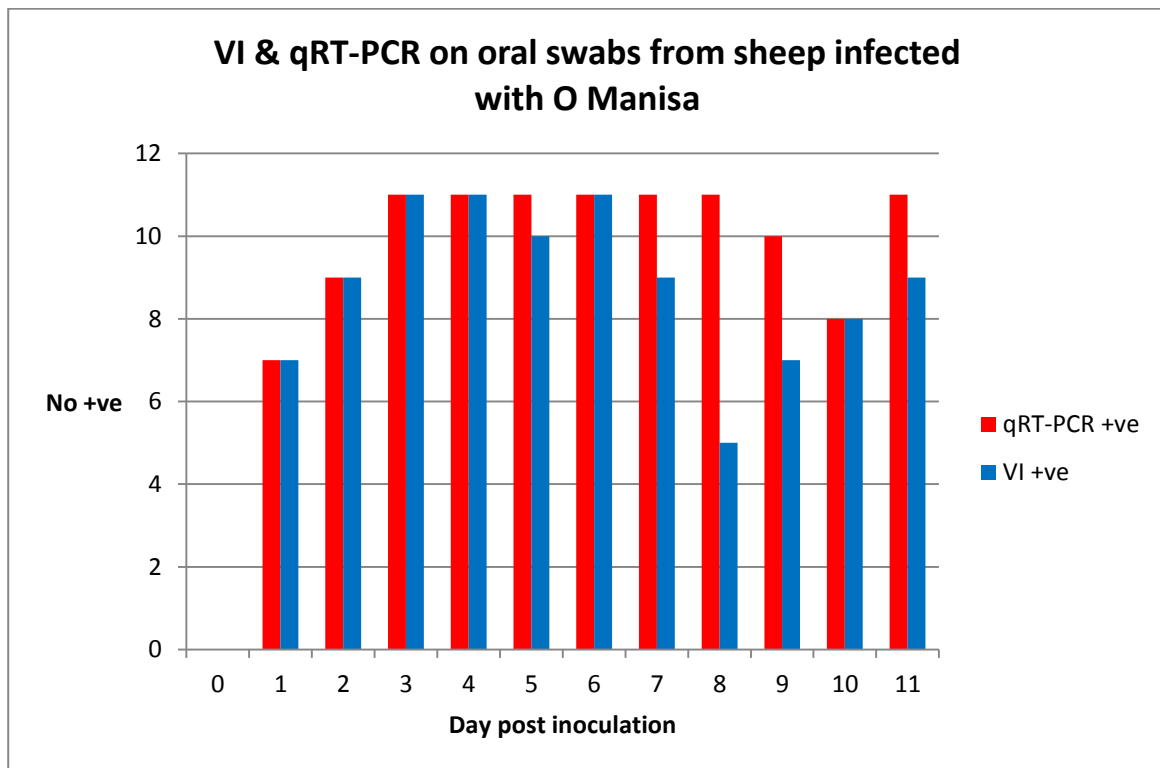


Figure 3: Detection of FMDV in oral swabs of sheep infected with FMDV serotype O Manissa.

4.3 qRT-PCR – Pooled samples

The main objective of sample pooling is to reduce the number of samples to be tested but must be practical, cost effective and must not significantly compromise analytical sensitivity. To determine the impact of different pool sizes and stage of infection on analytical sensitivity, pools were created by the inclusion of single positive samples collected at different stages of infection.

4.3.1 Pooling strategy

There were several options to undertake sample pooling (or sample dilution). These included dilution of the samples in a sample buffer, test diluent or in other samples. As the outcome of testing samples in pools could be affected adversely by components of the sample matrix, a collection of samples taken prior to infection was pooled and used as the diluent. These studies were undertaken with the samples collected from 20 sheep that had been infected with FMD virus “Asia 1”. Viral transport medium collected from each of the sheep immediately prior to infection was pooled to provide a large volume of “diluent” for later investigations. To investigate the differences at different stages of infection, individual samples collected at Day 2 or Day 28 after infection were diluted and tested. The viral RNA level in each individual sample was known from earlier testing and was used as a guide to the range of dilutions that were prepared for each sample. Samples collected on Day 2 after infection were tested at dilutions of 1/3, 1/5 and 1/10 (ie equivalent to pools of 3, 5 or 10 samples). As testing of individual samples had shown lower levels of viral RNA at Day 28, these samples were only tested at dilutions of 1/3 and 1/5 (ie equivalent to pools of 3 or 5 samples).

4.3.2 qRT-PCR – Oral Swabs

For the samples collected on Day 2 samples, 19/20 gave positive results (Ct values <40) when tested undiluted. When tested at dilutions of 1/3, 1/5 and 1/10, 18 of the 19 positive samples gave positive results at each of these dilutions (Table 1). A single sample gave a positive result at a 1/5 dilution but was negative at the 1/3 and 1/10 dilutions. Unfortunately due to a significant logistics issue, it was not possible to retest this sample to establish whether these were real differences or were human error during pipetting. Based on the reactivity of this sample when tested undiluted, the result for the 1/5 dilution has given an expected result and it is almost certain that this sample should have given a definite positive result at the 1/10 dilution. In an optimised qRT-PCR assay it would be expected that a 1/10 dilution would result in an increase of reactivity (lower virus concentrations give higher Ct values) of about 3.5-4.0 Ct units – in this case an endpoint of about 35-36, well below the limit of detection.

When the day 28 samples were tested, 16/20 undiluted samples gave positive results. Fifteen of these positive samples were tested at dilutions of 1/3 and 1/5, all giving positive results (Table 2). Based on the levels of reactivity observed, some of these samples could also be expected to give positive results at a 1/10 dilution.

For all of the samples tested, the exogenous (Xeno) internal control RNA performed to expectation with acceptable sensitivity and was detected at levels that indicated that RNA extraction had been efficient and that there was no apparent evidence of inhibitors of PCR.

Table 1: qRT-PCR results (as Ct values) for oral swabs from sheep experimentally infected with FMDV Asia 1. The undiluted swabs were collected on Day 2 after infection and were diluted in pooled viral transport medium collected from sheep prior to infection

Sheep	Day 2 post infection			
	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	NT	NT	NT
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

Table 2: qRT-PCR results (as Ct values) for oral swabs from sheep experimentally infected with FMDV Asia 1. The undiluted swabs were collected on Day 28 after infection and were diluted in pooled viral transport medium collected from sheep prior to infection (NT=not tested)

Sheep	Day 28		
	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

4.4 High throughput qRT-PCR

Although the primary objective of this project was to evaluate the suitability of oral swabs as a sample to undertake FMD diagnosis and surveillance, there was an expectation that, should oral swabs be a suitable sample type, this would facilitate large scale testing. Over a 3 day period, 3 laboratory staff were able to complete testing of more than 1500 samples. This was achieved during the course of 'normal working hours' and utilising a system that was not fully configured or optimised for large scale high through-put testing. Although there is considerable scope to streamline the systems that were used, it is clear that the collection of oral swabs and the test systems used would facilitate large scale high through-put testing of the order that was achieved at EMAI (up to 3,000 samples in a day) during the equine influenza outbreak. If samples were pooled, this would represent a capacity of 15-30,000 samples per day. Even without pooling of samples, such a capacity would exceed any predicted needs for managing a response to an FMD outbreak.

4.5 Virus inactivation studies

Although there has never been an incident associated with the 'escape' of FMDV from a diagnostic laboratory, because of the high consequences of an 'escape' of this virus, most laboratories that handle FMDV have rigorous protocols to work with the virus. Where assays such as qRT-pCR are used, there is no need for 'live' infectious virus. Consequently, samples are treated to inactivate the virus. The standard German protocol involves treatment of the samples with a lysis buffer followed by heating at >70°C. The heating step is included because the lysis buffer used leaves a very low level of infectious virus. However, the results of the current project would suggest that this sample treatment protocol may be harsh and may be adversely affecting assay performance. This conclusion was reached because some of the results of the dilution series for the pooling studies are not logical. Specifically, samples that appear to have low levels of RNA (i.e. samples with high starting Ct values) give similar results when diluted 10 fold (rather than showing results that are 3.5 - 4 units higher). This suggests that higher concentrations of RNA are likely to be present than are being detected.

While the results of this project are outstanding, there may be scope for further improvements. These should include an evaluation of the capacity of alternative sample lysis buffers to fully inactivate FMDV, establishing whether heat treatment is required and, if so, whether alternative protocols might be suitable.

5 Discussion

5.1 Application of real time PCR to FMDV diagnosis and surveillance

5.1.1 Oral swabs

The principal objective of this project was to assess the suitability of oral swabs as an alternative sample for FMDV diagnosis and surveillance. The capacity to detect FMDV in oral swabs exceeded expectations, both in terms of the proportion of swabs that gave positive results after the first 10-14 days post-infection as well as the duration over which positive results were obtained. The detection of FMDV during a surveillance program is undertaken at a herd rather than individual animal level. Sixty five percent of samples were still positive at 30 days post infection, when the collection of samples ceased. Based on the

level of reactivity in these samples, it is likely that positive results would be detected for many more days. Consequently, with the collection of a small number of samples, it is almost certain that FMDV would be readily detected when testing oral swabs. As oral swabs are both an easy sample to collect and to test (with minimal processing prior to RNA extraction), they have proven to be an ideal sample for surveillance in sheep, a species in which FMDV can be difficult to detect.

5.1.2 Testing of plasma samples

Testing undertaken during this project has clearly demonstrated the higher sensitivity of qRT-PCR compared to virus isolation. This was shown with both oral swabs and when testing plasma samples. For plasma, there were more than twice as many samples that gave positive results. Nevertheless, compared to oral swabs, FMDV was detected for a much shorter time in plasma. When the logistics of collecting blood samples from sheep are also taken into consideration, it is clear that, although serum or plasma is needed for serological surveillance, plasma would not be recommended as the preferred sample for FMDV detection.

5.1.3 Pooling of samples

The pooling studies completed clearly demonstrate that it should be possible to test pools of samples collected at different stages after animals have been infected with confidence. Unlike this project, in an outbreak, animals are likely to be sampled at different times after the onset of infection. Consequently, it is likely that pools of 10 samples could be used with success. If a more conservative approach was needed, it is clear that pools of 5 samples will readily allow the detection of FMDV RNA, even when animals have been infected for up to a month previously. When combined with the collection of oral swabs, the use of qRT-PCR to test pools of samples will support rapid large scale surveillance for FMDV in sheep.

5.1.4 Virus inactivation

While diagnostic laboratories have not been implicated in the spread of FMDV, perceptions of risk remain and there is a need to exercise 'due diligence' by handling non-infectious material where possible. Although the levels of infectious virus remaining after treatment with the German sample lysis buffer are extremely low, and probably too low to infect an animal, heat treatment was implemented to ensure complete inactivation. Although the qRT-PCR assay generally performed well, there are strong indications that the additional heat treatment may have reduced the efficiency of the qRT-PCR. It would be beneficial to assess alternative lysis buffers to establish whether virus infectivity may be abolished without further treatment. Due to time constraints, it was not possible to evaluate the efficacy of the buffer routinely used at EMAI but this should be pursued if the opportunity arises. Preliminary investigations could be undertaken using related viruses as a proxy for FMDV.

5.2 Achievement of objectives

The objectives of this project and the associated outcomes were as follows:

- 1 To confirm the capacity of quantitative real-time PCR (qRT-PCR) to detect FMDV in oral fluids and oral swabs from sheep: **Successfully achieved**

- 2 Define the onset and duration of detection of FMDV in oral fluids, swabs and serum: **FMDV RNA detected in samples over an extended period, from Day 2 after infection through to at least Day 30.**
- 3 Estimate the level of pooling of samples that may be achieved without compromising the capacity to detect FMDV in a flock. **Pool sizes ranging from 5-10 samples can be used for detection of FMDV in a flock over a period of at least one month after infection.**

6 Conclusions/recommendations

These studies have shown that FMD viral RNA can be confidently detected in sheep by the collection of oral swabs and tested with a real time PCR assay. This approach adds a new dimension to the detection of FMDV in sheep and will facilitate surveillance in the event of a disease outbreak. As the virus levels in sheep are low compared to cattle and pigs, it is expected that this sampling strategy can also be readily applied to monitoring of these species. The capacity to rapidly detect FMDV without amplifying infectious virus reinforces plans to undertake FMDV surveillance testing at a regional level.

The evaluation of alternative sample lysis buffers is warranted to ensure that virus is inactivated to the greatest level possible without having any adverse effects on assay sensitivity or specificity.

The outcomes of this project have already been conveyed to the national FMDV Vaccine Evaluation & Advisory Group and also to the leading scientists from laboratories that form the national emergency response network (LEADDR). However, it is important that these results are also conveyed to Animal Health Australia to ensure inclusion of this information in the AUSVETPLAN for FMDV.

7 Acknowledgements

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