Pathogenic Characteristics of the Korean 2002 Isolate of Foot-and-Mouth Disease Virus Serotype O in Pigs and Cattle

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Summary

Experimental infection of susceptible cattle and pigs showed that the O/SKR/AS/2002 pig strain of foot-and-mouth disease virus (FMDV) causes an infection that is highly virulent and contagious in pigs but very limited in cattle. Pigs directly inoculated with, or exposed to swine infected with, strain O/SKR/AS/2002 showed typical clinical signs, including gross vesicular lesions in mouth and pedal sites. In addition, FMDV was isolated from, and FMDV genomic RNA was detected in, blood, serum, nasal swabs and oesophageal—pharyngeal (OP) fluid early in the course of infection. Antibodies against the non-structural protein (NSP) 3ABC were detected in both directly inoculated and contact pigs, indicating active virus replication. In contrast, the disease in cattle was atypical. After inoculation, lesions were confined to the infection site. A transient viraemia occurred 1 and 2 days after inoculation, and this was followed by the production of antibodies to NSP 3ABC, indicating subclinical infection. No clinical disease was seen, and no antibodies to NSP 3ABC were present in contact cattle. Additionally, no virus or viral nucleic acid was detected in blood, nasal swab and OP fluid samples from contact cattle. Thus, the virus appeared not to be transmitted from infected cattle to contact cattle. In its behaviour in pigs and cattle, strain O/SKR/AS/2002 resembled the porcinophilic FMDV strain of Cathay origin, O/TAW/97. However, the latter, unlike O/SKR/AS/2002, has reduced ability to grow in bovine-derived cells. The porcinophilic character of O/TAW/97 has been attributed to a deletion in the 3A coding region of the viral genome. However, O/SKR/AS/2002 has an intact 3A coding region.

Introduction

Foot-and-mouth disease virus (FMDV), a member of the Aphthovirus genus within the Picornaviridae, is the causative agent of an economically important disease of cloven-hoofed animals, and the single most important constraint to trade in live animals and animal products. FMDV is a positive-stranded RNA virus (~8.5 kb), which occurs as seven distinct serotypes (A, O, C, SAT1, SAT2, SAT3 and Asia 1). The Pan-Asia type O topotype has caused great devastation in Asia in recent years (Sakamoto et al., 2002; Knowles and Samuel, 2003; Feng et al., 2004; Wee et al., 2004).

The Republic of Korea had been free from foot-and-mouth disease (FMD) since 1934 until outbreaks were reported in 2000. These outbreaks occurred in cattle farms only. Eradication, at a cost of $233.6 million, was achieved by slaughter, quarantine, prohibition of livestock transport, and vaccination around infected areas. Korea then remained free from FMD...
until 2002, when a further outbreak on a pig farm oc-
curred, spreading to 14 additional pig farms and one
dairy farm, with serious implications for recently re-
sumed pork exports worth $400 million annually.

During the 2002 outbreak, field veterinarians
observed severe vesicular lesions in pigs on all farms,
but such lesions were described in cows only on one
farm. Comparison of genomic sequences of the 2000
and 2002 Pan-Asian isolates (O/SKR/2000 and O/ SKR/AS/2002) failed to identify any obvious differ-
ence that might have accounted for the difference
observed in the pathogenic properties of the two
strains (Oem et al., 2004). To understand a difference
in pathogenicity for pigs and cattle of an FMDV
strain, it is necessary to examine infection in each
host. The 3A protein of FMDV plays a role in viral
virulence, and alteration or deletion of this gene is
associated with the reduced ability of some strains of
FMDV to cause disease in cattle (Mason et al.,
2003; Pacheco et al., 2003; Grubman and Baxt,
2004). Protein 3A is a viral non-structural (NS) pro-
tein containing 153 amino acids and its role in
FMDV replication is unclear. While this protein is
generally highly conserved in most FMDV strains
examined to date, an outbreak of FMD in Taiwan
in 1997 was caused by a virus carrying a deletion in
3A (codons 93–102) in addition to a highly mutated
region downstream of the deletion (Beard and Mason,
2000). This new strain, a member of the Cathay
topotype (Huang et al., 2000), caused severe disease
in swine but no signs of infection in cattle. It is note-
worthy that known 3A-deletions were associated
with the attenuation of FMDV strains by egg-adapta-
tion for the development of vaccines for cattle in
South America (Giraudo et al., 1990). The 3A region
of several Asian isolates was recently examined and
some were found to harbour a second deletion at
codons 133–143 (Knowles et al., 2001).

The aim of this study was to investigate the charac-
teristics of FMDV O/SKR/AS/2002 and its pathoge-
nicity for cattle and pigs.

Materials and Methods

FMDV Strains

Strain O/SKR/AS/2002 (Oem et al., 2004) was origi-
nally isolated in pig kidney (IBRS-2) cells from the
epithelium of an infected pig in Anseong (AS) Pro-
vince, Korea in 2002.

In-vitro Studies of Strain O/SKR/AS/2002 in Different Cell

IBRS-2 cells (passage 200), bovine kidney cells
(BKLF; passage 125) and fetal bovine kidney cells
(FBK; passage 3) were seeded in 96-well cell culture
plates, each well receiving 100 μl of a suspension con-
taining 4 × 10^3 cells/ml. The plates were shaken for
30 s and then incubated at 37°C in a humidified 5%
CO2 incubator. Confluent cells were infected with
serial 10-fold (10^{-1}–10^{-8}) dilutions of O/SKR/AS/
2002 suspension. Replicates of 10 wells (1st to 10th
column) in a 96-well plate were used for each virus di-

tution (25 μl/well) and the plates were incubated at
37°C in a humidified 5% CO2 incubator for 60 min.
To each well were then added 100 μl of D-MEM
medium containing fetal bovine serum 5%, before in-

cubating the plates at 37°C in a humidified 5% CO2
atmosphere for 3 days. For each plate, the number of
wells at each dilution with (+) or without (−) a cyto-
pathogenic effect (CPE) was recorded. The 50%
endpoint titre of the virus was determined to calculate
the 50% tissue culture infectious dose (TCID_{50}.

RNA Isolation and Nucleotide Sequence of
the 3A Coding Region

Total RNA was directly extracted from O/SKR/AS/
2002-infected IBRS-2 cells (the first passage from the
original isolate) with Trizol reagent (Life Technologies,
Gaithersburg, MD, USA). It was then used as a tem-
plate for first-strand synthesis of cDNA, with Super-
Script First-Strand Synthesis System for RT-PCR
(Life Technologies) and random hexamers as primers.
The polymerase chain reaction (PCR) amplification
was carried out with AdvanTaq DNA Polymerase
(Clontech, Palo Alto, CA, USA) and the specific oligo-
nucleotide pair P797/P799 (5'-TGAAGAGC GG-3'/5'
GCAGGTAAAGTG-3') encoding the non-structural
Δ2C/3A/B1B2B3/3C/Δ3D region of O/SKR/AS/2002
(GenBank accession number AY312589). PCR prod-
ucts were purified from agarose gels with Qiagen col-
umns (Qiagen, Valencia, CA, USA) and sequenced
directly with selected primers and the ABI PRISM Big-
Dye Terminator Cycle Sequencing Ready Reaction
Kit v3.0 (Applied Biosystems, Foster City, CA, USA),
followed by resolution on an ABI3100 sequencer. The sequence data were further analysed
with the MacVector™ program.

Animal Experiment

All animal manipulations were performed by proce-
dures approved by the Animal Care and Use Com-
mittee of the Plum Island Animal Center. A suspen-
sion of strain O/SKR/AS/2002 (10^7 TCID_{50}/
ml) was used to inoculate one Holstein calf (no. 2),
weighing 250–300 kg, at six intradermal lingual sites
(0.1 ml per site). This animal was immediately
housed in a room with four other Holstein cattle
(nos 1, 3–5) of similar size. One Yorkshire pig (no. 61), weighing 25–30 kg, was infected with the same inoculum in the front heel bulb at eight intradermal sites (40 μl per site). This animal was housed with five other pigs (nos. 61, 63–66) in a room separate from that used for the cattle. For each animal, the clinical signs and lesions were recorded daily. Blood (collected in EDTA-treated tubes), serum, nasal swabs, and oro-pharyngeal (OP) fluid were collected daily from 0 to 14 days post-inoculation (DPI) or days post-exposure (DPE), then once every 7 days for the next 6 weeks (i.e., until day 56 DPI/DPE). Pig 61 was killed by the intravenous administration of sodium pentobarbital (Fatal Plus, Vortech, Dearborn, MI, USA) at 4 DPI, after epidermal lesions had fully developed, for complete necropsy and histopathological examination.

**Scoring of Clinical Signs**

The clinical scores for cattle were determined as follows: tongue lesion beyond inoculation site = 1; mouth lesion other than tongue = 1; lesion on nostril = 1; one lesion per foot = 1; and two or more lesions per foot = 2. The maximum score for cattle was 11. Clinical scores for pigs were based on the sites containing FMD lesions (vesicular lesions, erosion of epithelium, and blanching of the coronary band). One point was awarded for each affected digit, and for each of three sites (tongue, snout, and lower lip) bearing one or more vesicles. The maximum lesion score for pigs was 19. The scores of each pig were recorded daily until the vesicles at all sites had started to heal (Pacheco et al., 2003).

**Histopathology**

Pig 61 was killed at 4 DPI and a necropsy was performed. Samples of epidermis at the coronary band of all four digits of all four feet, and samples of snout, lip, tongue, heart, lung, liver, kidney and spleen were immersed in 10% neutral buffered formalin, processed by routine methods and embedded in paraffin wax, and sections (5 μm) were mounted on glass microscope slides, stained with haematoxylin and eosin (HE), “coverslipped” and examined.

**Isolation of Virus from Blood, Serum, Nasal Swabs and OP Fluid**

IBRS-2 cells (passage 193) were seeded in T-25 flasks (Corning Inc., Corning, NY, USA). Confluent cells were infected with 200 μl of EDTA-treated blood, nasal swab samples or OP fluid samples and allowed to adsorb for 60 min at 37°C in a 5% CO₂ incubator. Six milliliter of medium (see above) were added to each flask, which was then examined daily for a CPE, characterized initially by rounded cells, and eventually by the entire destruction of the cell monolayer. In the second passage of virus isolation, 200 μl of fluid from previously inoculated T-25 flasks were added to confluent IBRS-2 monolayers in individual wells of 24-well plates (Corning), which were then examined daily. Based on the appearance of CPE or otherwise, the results were recorded as initial virus isolation positive or negative. For each blood sample, 5 ml were collected in EDTA tubes. Nasal swabs were immersed in 5 ml of Hepes medium containing antibiotic. OP fluid was collected into 10 ml of the same medium and “vortexed” vigorously. A 500-μl volume of each OP sample in Hepes medium was transferred into a 1-ml Eppendorf tube for centrifugation in an Eppendorf/centrifuge 5415 (Brinkmann Instruments Inc., Westbury, NY, USA) at 14 000 rpm for 2 min. Supernate (200 μl) was utilized for virus isolation. There were control T-25 flasks (the first passage) and control 24-well plates (the second passage) for each inoculation procedure.

**Real-time PCR (RT-PCR) on Serum, Nasal Swabs and OP Fluid**

For all samples, RNA was extracted with a MagMax-96 viral RNA isolation kit (Ambion, Austin, TX, USA). Briefly, 25 μl of sample were added to 101 μl of lysis/binding mix (Lysis/Binding solution 50 μl, Poly[A] RNA 1 μl, 100% isopropanol 50 μl) in a 96-well round-bottom microtitre plate (Evergreen Inc., Los Angeles, CA, USA). Next, 20 μl of Beads/ Binding Mix (RNA binding beads 4 μl, nuclease free water 4 μl, Lysis/Binding solution 6 μl, and 100% isopropanol 6 μl) were added to each well. The plate was gently shaken on a Titer Plate Shaker (Lab-Line Instruments Inc., Melrose Park, IL, USA) at dial position 5 for 4 min. Supernate (200 μl) were pelleted for 2 min on a 96-well magnet (Ambion) and the supernate was removed. To each well, 100 μl of Wash Solution I Mix (Wash Solution I [Ambion] 50 μl, 100% isopropanol 50 μl) were added, and the plate was shaken at dial position 5 for 30 s. The beads were then pelleted (1 min) and the supernate was removed. The beads were washed twice with 100 μl of Wash Solution II Mix per well (Wash Solution II [Ambion] 30 μl, 100% ethanol 180 μl), shaken at dial position 5 for 30 s, and the supernate was removed after pelleting the beads for 30 s. The beads were shaken vigorously at dial position 9 for 2 min to dry them. They were then mixed with Elution Solution (Ambion) 25 μl/well and shaken at dial position 9 for 4 min. Finally, the beads were pelleted for 2 min.
and the RNA was transferred to a storage plate and frozen at −70°C.

Reverse transcriptase real-time PCR (rRT-PCR) was performed on the extracted RNA. Primers and probe targeting the 3D region of FMDV were designed previously (Callahan et al., 2002). Reagents from the EZ rTth Kit (Applied Biosystems, Foster City, CA, USA) were used to prepare the master mix according to the guidelines of the manufacturer for individual component concentrations. The final rRT-PCR mixture (25 μl per sample) consisted of

\[
1.2 \text{ mM dNTPs, 2.5 U rTth polymerase, and 50×}
\]

Rox reference dye (Invitrogen, Carlsbad, CA, USA). The rRT-PCR was performed on the Stratagene Mx4000 thermocycler as follows: an initial step of 60°C for 60 s, followed by 45 amplification cycles of 95°C for 30 s and 65°C for 60 s (Callahan et al., 2002).

**Measurement of Non-structural Protein (NSP) Antibodies**

The 3ABC indirect enzyme-linked immunosorbent assay (I-ELISA) was modified from Meyer et al. (1997). Briefly, 3ABC protein was produced in a baculovirus system. Crude 3ABC antigen and negative control antigen in 8 M urea were coated in triplicate on Immulon 2 plates (Dynex Technologies, Chantilly, VA, USA) for a minimum of 24 h at 4°C. Test serum samples were automatically converted into arbitrary values by a linear equation (Softmax Pro Program; Molecular Devices, Sunnyvale, CA, USA). Sera from an FMDV-infected and a non-infected animal were run on each plate as positive and negative controls, respectively. The arbitrary cut-off value of this test was 10 in cattle and 13 in pigs.

**Results**

**Replication of Strain O/SKR/AS/2002 in Different Cell Lines**

CPEs were produced in all three cell lines (BKLF, FBK and IBRS-2). The TCID50 values of the same stock of virus (first passage in IBRS-2 cells of the original isolate) in BKLF, FBK and IBRS-2 cells were 10⁻³.³, 10⁻⁶.⁰ and 10⁻⁶.², respectively, i.e., approximately one log higher in BKLF cells than in IBRS-2 or FBK cells. No CPE was observed in uninfected control cells.

**Comparison of 3A Sequences**

To map changes in the NSP 3A, RNA was isolated from FMDV-infected IBRS-2 cells and subjected to RT-PCR. The PCR was designed to amplify a 3412-bp region consisting of a fragment of 2C, all of 3A/3B/3D⁰/3C⁰, and a partial sequence of 3D⁰. The PCR product was extracted from the agarose gel and sequenced. The sequence data showed no deletions in 3A and accorded with a recently deposited sequence of the L-fragment of O/SKR/2002 sequence (GenBank accession number AV312589), except for one amino-acid change (T116A) found in codon 116 within the hypervariable region of FMDV 3A.

**Clinical Signs and Lesion Score**

**Cattle.** At 1 DPI, calf 2 showed increased salivation and blanching in five of the six intradermal lingual sites of inoculation. At 2 DPI, salivation was still present, erosions had started to develop on the five affected lingual sites, and the rectal temperature had reached 39.4°C. At 3 DPI, five discrete, oval, green lesions (indicative of epithelial necrosis and shallow erosion) were observed on the tongue (Fig. 1), corresponding to virus injection sites, and the rectal temperature was 39.1°C. At 4 DPI, the erosions persisted but the rectal temperature had decreased to 38.7°C. No vesicle was observed on any externally visible epithelium of this animal throughout the experiment. The injection site lesions were fully healed by 8 DPI. None of the contact cattle (nos 1, 3–5) showed any clinical signs or vesicular lesions throughout the experiment.

**Pigs.** At 1 DPI, the inoculated pig (no. 61) showed blanching of all eight injection sites in the heel bulb. During day 2, vesicles started to develop (1) at the injection sites, (2) on the right edge of the tongue, and (3) on all feet, including the coronary bands, heel bulbs, and interdigital spaces. At 4 DPI, rupture of vesicles and epithelial necrosis on the heel bulbs
were observed (Fig. 2). At 3 DPI, the erosion score of pig 61 had increased to 15 (Fig. 3), and at 4 DPI this animal was killed. Three contact pigs (62, 63 and 65) showed foot lesions at 3 DPE and the remainder (64 and 66) at 4 DPE. At 7 DPE, the lesion scores for contact pigs 62, 63, 64 and 66 had reached 19, 18, 17 and 19, respectively. Pig 65 had a lesion score of 10 at 7 DPI (Fig. 3). All contact pigs were pyrexic from 4 to 6 DPE, the temperature at 6 DPE being $>40\degree$C.

**Histopathological Findings in Pig 61 at 4 DPI**

**Coronary band and digits of all four feet.** Microscopical changes observed in haired skin sections of the feet (also snout and lip; see below) included moderate to marked acute vesicular, erosive and ulcerative dermatitis, with epithelial degeneration and necrosis, intraepithelial microabscesses, oedema, and secondary bacterial infection. Individual and multifocal micro-vesicles and large coalescing vesicles, some in the early stages of rupturing, were observed. There were multiple epidermal erosions and ulcerations, some of which resulted in colonization of exposed dermal collagen bundles by coccal bacteria. In all sections, there was degeneration, coagulative necrosis and dissociation of epithelial cells (individual cells and aggregates) within the stratum spinosum, characterized by shrunken hypereosinophilic cytoplasm, condensation of chromatin, and pyknosis. Perinuclear cytoplasmic vacuolation (intracellular oedema) of epithelial cells at the margins of the lesions was often noted, as also was infiltration of the epidermis by viable and degenerate neutrophils, with formation of micropustules. Small intact vesicles contained homogeneous eosinophilic material (proteinaceous fluid); larger vesicles consisted of rarefied areas containing large numbers of neutrophils, smaller numbers of macrophages, and variable amounts of eosinophilic fibrillar material (fibrin) (Fig. 4). Spinocytes (individual cells and rafts) could be seen desquamating into the lumina of many vesicles. Within areas of epithelial erosion and ulceration, the adjacent intact epidermis and subjacent dermis contained large amounts of viable and degenerate neutrophils, and pyknotic and karyorrhectic debris. The dermis was often infiltrated in a multifocal and perivascular pattern by moderate numbers of macrophages admixed with smaller numbers of neutrophils and lymphocytes. Endothelial cell hypertrophy was noted occasionally, characterized by plump endothelial cell nuclei protruding into the vascular lumen. There was often extensive rarefaction and expansion of the papillary dermis, seen as a clear space (oedema); this change occasionally extended into the reticular dermis (Fig. 5).

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**Fig. 1.** Tongue of calf 2 at 3 DPI. Five discrete foci of coagulative necrosis are seen, with mild superficial erosion of the epithelium. The lesions, which were confined to intradermointerlingual injection sites, were completely resolved by 8 DPI.

**Fig. 2.** Epithelial necrosis on heel bulbs of the left hind foot of pig 61 at 4 DPI.

**Fig. 3.** The lesion scores of pigs 61–66 on various days post-inoculation or post-exposure (DPI or DPE).
In one section of snout there was necrosis of epithelial cells in the superficial stratum spinosum, with rounding and dissociation of cells. In another section, within the same region of the epidermis, a moderately sized intact vesicle was present, partly filled by neutrophils, lymphocytes, proteinaceous fluid, fibrin and a few erythrocytes. Increased clear space between epithelial cells (spongiosis) and intracellular oedema was noted adjacent to the vesicle.

**Lip.** There was an extensive ruptured vesicle within the haired skin of the lip, with formation of a large flap of necrotic epithelium. Multiple discrete and coalescing epidermal micropustules were also present, as was extensive intracellular and intercellular oedema. Hydroptic degeneration of epithelial cells of hair follicles was seen occasionally. Rarefaction of the dermis and dilatation of dermal lymphatic vessels (oedema) accompanied mild lymphohistiocytic infiltrates. No alterations were noted in the non-keratinizing mucosal epithelium.

**Heart.** In a section of the left ventricle, mild multifocal lymphohistiocytic myocarditis was observed, characterized by scattered small aggregates of lymphocytes and histiocytes within the myocardial interstitium and in the subendocardial connective tissue (Fig. 6). Mild cardiac myofibre degeneration, characterized by granular fragmentation of the sarcoplasm, was also noted.

**Other tissues.** No significant microscopical lesions were noted in sections of the tongue, lung, liver, kidney or spleen.

**Virus Isolation (Cattle and Pigs)**

In calf 2, FMDV was first isolated from the whole blood at 1 DPI (Table 1). Two days later it was also isolated from OP fluid, but no virus was isolated from nasal swab samples. In contact cattle, no virus was isolated from blood, nasal swab or OP fluid samples collected at any timepoint. In pig 61, FMDV was...
isolated from blood at 1 DPI, and the viraemia persisted for an additional 2 days before the animal was killed; virus was also isolated from nasal swab and OP fluid samples from this donor pig at 2–3 DPI. FMDV was isolated from blood, nasal swab, and OP fluid samples of various contact pigs from 2 to 6 DPE. No virus was isolated from blood or nasal swabs of pig 65, or from OP fluid of pig 64 (Tables 1–3).

**Real-time PCR (Cattle and Pigs)**

The inoculated calf (no. 2) gave positive results with blood collected at 4 DPI and with OP fluid samples collected at 2–6 DPI. Contact cattle gave negative results with all serum, nasal swab, and OP fluid samples. The inoculated pig (no. 61) gave positive results with blood, nasal swab and OP fluid samples collected at 2–3 DPI. Contacts gave positive results with blood, nasal swab and OP fluid samples from 3 to 8 DPE, 2 to 8 DPE, and 2 to 14 DPE, respectively (Tables 1–3).

**NSP 3ABC Antibody Test**

Calf 2 developed antibodies to 3ABC (detectable by IELISA) at 8 DPI; the titre reached a peak at 28 DPI and then started to decline. No 3ABC antibodies were detected in any of the contact cattle from 0 to 56 DPE. The 3ABC antibodies of pigs 62, 63, and 64 increased significantly at 8, 9, and 11 DPE, respectively, and all reached a peak at 14 DPE before starting to decline. The 3ABC antibodies of pig 66 showed a significant increase at 11 DPE and reached a peak at 21 DPE before declining. Pigs 62–64 and 66 had antibody titres over 100% arbitrary values at their maximum; however, pig 65 showed a significant increase in 3ABC antibody titre at 13 DPE but did not reach 40% arbitrary values even with the highest titre at 21 DPE (Figs. 7 and 8).

**Discussion**

The experiment showed that strain O/SKR/AS/2002 was highly virulent and contagious for pigs, causing vesicles in the mouth and on the feet. Active viral replication in all six infected pigs (one inoculated and five in-contact) was demonstrated by (1) isolation of FMDV in cell culture from whole blood, nasal swab and OP fluid samples, (2) the presence of FMDV nucleic acid in serum, nasal swabs and OP fluid, (3) the presence of gross lesions consistent with FMD, and (4) the presence of antibodies against the NSP 3ABC in serum.

In contrast, the disease in cattle was atypical, no lesions developing in inoculated or in-contact cattle, other than at the injection sites in the single
Table 2

Virus isolation (VI) and real-time polymerase chain reaction (PCR) of nasal swabs collected on various days post-inoculation (DPI) or days post-exposure (DPE) from cattle and pigs

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* Calf 2 and pig 61 were inoculated with strain O/SKR/AS/2002.

† The VI results are expressed as first passage/second passage in IBRS-2 cells, and as FMDV isolated (+) or not isolated (−).

‡ Pig 61 was killed for necropsy and histopathological examination at 4 DPI, i.e., when epidermal lesions were fully developed.

Table 3

Virus isolation (VI) and real-time polymerase chain reaction (PCR) of OP fluid samples collected on various days post-inoculation (DPI) or days post-exposure (DPE) from cattle and pigs

<table>
<thead>
<tr>
<th>DPI or DPE</th>
<th>Calf 2*</th>
<th>Calf 1</th>
<th>Calf 3</th>
<th>Calf 4</th>
<th>Calf 5</th>
<th>Pig 61*</th>
<th>Pig 62</th>
<th>Pig 63</th>
<th>Pig 64</th>
<th>Pig 65</th>
<th>Pig 66</th>
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<tr>
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<td>VI</td>
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</table>

* Calf 2 and pig 61 were inoculated with strain O/SKR/AS/2002.

† The VI results are expressed as first passage/second passage in IBRS-2 cells, and as FMDV isolated (+) or not isolated (−).

‡ Pig 61 was killed for necropsy and histopathological examination at 4 DPI, i.e., when epidermal lesions were fully developed.
inoculated animal (calf 2). Although 3ABC antibodies were detected in calf 2, the only lesions that occurred were erosions at the injection sites on the tongue. These were accompanied by weak viraemia. In the contact cattle, neither 3ABC antibodies nor vesicles occurred; furthermore, no virus could be isolated from whole blood, nasal swabs or OP fluid samples and PCR testing of serum, nasal swabs and OP fluid samples gave negative results. Thus, the experiment demonstrated failure of this virus to spread to the contact cattle over an 8-week period.

Strain O/SKR/AS/2002 is phenotypically similar to the porcinophilic strain (O/TAW/97) of Cathay virus. *In-vivo* studies of FMDV strain O/TAW/97 performed at the OIE World Reference Laboratory for FMD at Pirbright, UK showed that this virus did not cause clinical disease in cattle even by direct inoculation in the tongue (Dunn and Donaldson, 1997).

Fig. 7. The 3ABC (NSP) antibodies of calfs 1–5 on various days DPI or DPE.

In *-vivo studies of O/TAW/97 demonstrated reduced ability to grow in bovine cell cultures (Giraudo et al., 1987; Sagedahl et al., 1987; Beard and Mason, 2000; Mason et al., 2003); however, O/SKR/AS/2002 grew efficiently in both bovine and porcine cells. The apparent inability of strain O/TAW/97 to replicate effectively in cattle is similar to that of Cathay lineage type O FMDVs co-circulating in Asia. The question arises as to whether the O/SKR/AS/2002 isolate sent to the Plum Island Animal Disease Center was related to the Cathay lineage of viruses and not to the Pan-Asia lineage. One of the genetic markers that distinguishes the two lineages can be found in the 3A viral protein sequence. Cathay viruses, which show restricted growth in cattle, possess a deletion in 3A protein (Sagedahl et al., 1987; Giraudo et al., 1990; Dunn and Donaldson, 1997; Yang et al., 1999; Beard and Mason, 2000; Huang et al., 2000; Grubman and Baxt, 2004). No such deletions are present in type O Pan-Asia isolates. Our sequence data, and a recently deposited sequence of the L-fragment of O/SKR/AS/2002, show no deletions in 3A. However, there is one amino-acid difference in codon 116 within the hypervariable region of FMDV 3A. One possible explanation for the difference is that the sequence deposited at GenBank was an original isolate and the O/SKR/AS/2002 we used for sequencing had been passaged once in IBRS-2 cells. The results, including the mild disease produced in the cattle, are consistent with the virulence of the Pan-Asia topotype and further indicate that the 3A deletion is not the only genetic marker related to host-range restriction.

The pathological findings in pig 61 were similar to those reported by Alexandersen et al. (2003). Although no gross lesions were observed in the heart, mild multifocal lymphohistiocytic myocarditis in a section of the left ventricle and mild cardiac myofibre degeneration were seen microscopically. In contact pigs, the occurrence of virus in OP fluid and nasal mucosa was followed by viraemia (Tables 1–3). Some studies have suggested that the initial site of FMDV replication is the pharynx (Burrows et al., 1981; Zhang and Kitching, 2001; Alexandersen et al., 2003) or in the respiratory tract (Brown et al., 1996). In the present study, viraemia generally accorded with lesion scores in pigs. After 6 DPI or DPE, no live virus was detected in blood, nasal swab or OP fluid samples. After 14 DPI or DPE, for all animals, no viral nucleic acid was detected in blood, nasal swabs or OP fluid. Alexandersen et al. (2003) reported that host immunity to FMD was primarily mediated by circulating antibodies, and that progressive clearance of virus from the circulation and a significant reduction from most organs through excretions and secretions were evident; in addition,
virus isolation in BTY cells and quantitative RT-PCR consistently failed to show that FMDV persisted in pigs for more than 3–4 weeks.

In this study, the combination of 96-well RNA extraction techniques and real-time PCR assisted in the rapid handling and processing of samples. It proved more efficient than the traditional RNA extraction procedure and RT-PCR, especially for dealing with large numbers of samples. RNA was extracted from sera, because a procedure for extracting RNA from blood on a 96-well plate had not yet been developed. The number of positive results obtained by virus isolation from EDTA-treated blood was greater than the number found by the use of PCR on serum samples. Possibly, FMDV may be cell-associated and therefore lost during serum preparation. It is also possible that serum samples contain an inhibitor of reverse transcriptase. Quan et al. (2004) reported that the sensitivity of FMDV isolation was 10–100 times less than that of RT-PCR. In the present study, the lack of sensitivity of FMDV isolation in cell culture may be similarly explicable. The results suggest that in pigs, nasal swab samples are preferable to EDTA-treated blood and OP fluid samples for virus isolation (Tables 1–3). Recent evidence suggests that viral replication is greater in the mucosa than in the lung (Oleksiewicz et al., 2001). In the present study, FMDV nucleic acid was detected for a longer period from porcine OP fluid samples by real-time PCR (14 DPE), than from serum or nasal samples (8 DPE).

In the 2002 Korean outbreak, two FMDV isolates (one from pigs and the other from cattle) of strain O/SKR/AS/2002 (Oem et al., 2004) appeared to be nearly identical on sequencing (data not shown). The strain used in the present experiment was from a pig. The data from 3A sequencing of pig-derived O/SKR/AS/2002 do not explain why this virus, which is highly infectious for pigs, has restricted growth properties in cattle. It is possible that more than one factor determines the porcinophilic character of this strain.

Acknowledgments

We thank Dr Michael LaRocco and Dr Maria Piccone at the Plum Island Animal Disease Center for providing FBK and BKLF cells, and for the in-vitro study. We also thank Dr. Marvin Grubman for his advice and review of the paper. We especially thank Dr Juan Lubroth who initiated this project. This study was supported by the National Veterinary Research and Quarantine Service, Ministry of Agriculture, Anyang, Republic of Korea.

References


[Received, April 23rd, 2007]

[Accepted, January 21st, 2008]