Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 30 (2003) 715–721
BBRC
www.elsevier.com/locate/ybbrc

Serotype and VP1 gene sequence of a foot-and-mouth disease virus from Hong Kong (2002)

Qian Feng,[a] Xi Chen,[a] Ou Ma,[a] Yingying Liu,[a] Mingxiao Ding,[a] Richard A. Collins,[b] Lung-Sang Ko,[b] Jun Xing,[b] Lok-Ting Lau,[b,c] Albert Cheung-Hoi Yu,[b,c] and Jianguo Chen[a,b,*]

[a] *Department of Cell Biology and Genetics, College of Life Sciences, Peking University, Beijing 100871, China*
[b] *Hong Kong DNA Chips Limited, 1805-6, 18/F, Lu Plaza, 2 Wing Yip Street, Kowloon, Hong Kong SAR, China*
[c] *Neuroscience Research Institute and Department of Neurobiology, Peking University, 38 Xue Yuan Road, Beijing 100083, China*

Received 31 January 2003

Abstract

The nucleotide sequence of the VP1 coding region of foot-and-mouth disease virus (FMDV) strain HKN/2002, isolated from a disease outbreak occurring in Hong Kong in February 2002, was determined and compared with the sequences of other FMDVs. The VP1 coding region was 639 nucleotides in length and encoded a protein of 213 amino acid residues. Comparison of the VP1 nucleotide sequence with those of other isolates indicated that HKN/2002 belonged to serotype O. A VP1-based sequence similarity tree of several South-east Asian FMDV-O isolates showed that HKN/2002 was most closely related to FMDV isolates found in Hong Kong from 1991 to 1999 and Taiwan in 1997. Comparison of the amino acid sequence of the major immunogenic region of HKN/2002 with that of the serotype O vaccine strain, O1/Manisa/Turkey/69, reveals significant similarity, indicating that current serotype O vaccines may offer some degree of protection against HKN/2002.

Keywords: Foot-and-mouth disease; VP1 protein; Serotype; Hong Kong

Foot-and-mouth disease (FMD) is probably the most contagious disease of cloven-hoofed animals [1,2]. The economic implications of a large-scale outbreak were amply demonstrated during the FMD epidemic occurring in the United Kingdom in 2001 [2]. The causative agent is foot-and-mouth disease virus (FMDV), an aphthovirus of the *Picornaviridae* family. The virus contains a single-stranded positive sense RNA genome about 7.2–8.4 kb in size. Seven major serotypes (A, Asia-1, C, O, SAT-1, SAT-2, and SAT-3) are known, and hundreds of isolates have been described and partially sequenced. In Hong Kong, FMD occurs regularly in pigs, especially in the winter months (December–March). The pig farming industry in Hong Kong comprises both domestically reared and imported animals. Live pigs are imported mainly from Mainland China, with small numbers of breeding stock imported from other countries. The Food and Environmental Hygiene Department (FEHD) checks imported live pigs and pork products at cross-border entry points. For imported live pigs, the FEHD checks the pigs for any clinical signs of animal disease, including FMD, and ascertains that the pigs are accompanied by valid health certificates certifying that they are free of animal disease. For importation of meat products from FMD-infected countries and regions, the FEHD checks the accompanying health certificate to see that the products are derived from animals free from diseases (including foot-and-mouth disease) and have passed ante and post-mortem examinations. In Hong Kong, FMD is controlled largely through vaccination of pigs with a multivalent vaccine prepared against strains likely to be encountered in the region (e.g., O1/Manisa/Turkey/69 and O-3039). Vaccination of susceptible animals against FMD is recommended but is not a mandatory requirement for the livestock industry in Hong Kong.

* Corresponding author. Fax: +86-10-6276-7044.
E-mail address: chenjg@pku.edu.cn (J. Chen).

0006-291X/03/$ - see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0006-291X(03)00250-X

Rapid identification of the causative agent is a key element of any control strategy [2]. Many different analytical techniques for the detection of viral nucleic acid, antigens, and host immune responses have been described, including nucleic acid sequence-based amplification (NASBA), real-time PCR, RT-PCR, enzyme-linked immunosorbent assay (ELISA), virus neutralisation, and complement fixation [1–3].

The VP1 structural protein, together with proteins VP2, VP3, and VP4, forms the capsid of the virus. The VP1 protein is encoded by 1D coding region of viral RNA, which is 627–639 bp long and produces a protein containing 209–213 amino acid residues, depending on the serotype studied. Amino acid residues 134–158 inclusive form a βG–βH loop structure forming the main immunogenic epitope of FMDV [4,5]. Mutation of residues 138–140 and 148–150 (serotype C numbering) in VP1 has been shown to influence FMDV antigenicity [6]. The mutation frequency of the FMDV VP1 gene is 1.6×10^{-3}–6.4×10^{-3} substitutions per nucleotide per year [7]. Comparison of the VP1 nucleotide and protein sequences of isolates obtained from different geographic regions at different times could provide evidence of the relatedness of individual isolates of FMDV. An evolutionary tree of FMDV-O strains derived from comparisons of a 165 amino acid residue sequence at the 3′-terminal of the VP1 gene revealed the existence of particular FMDV genotypes in India, which may help optimise the selection of vaccine strains to prevent future outbreaks [8].

The continued occurrence of FMD outbreaks in South-east Asia, e.g., Taiwan Province of China (1997), Malaysia (2001), South Korea (2002), Mongolia (2002), and Hong Kong SAR of China (2002), coupled with greater trade and tourism between infected and non-infected countries (regions), and the potential use of infectious agents of veterinary significance to cause economic damage in regions of intensive livestock farming by bioterrorists, requires the rapid identification of disease and implementation of control measures. A detailed knowledge of the molecular characteristics of the major immunogenic components of the virus will be helpful in tracking the evolution and origin of the virus and in developing specific diagnostic tests and protective vaccines. This paper provides the first description of the entire nucleotide sequence of the VP1 structural protein of HKN/2002, and compares it with the sequences obtained from previous FMD outbreaks in Hong Kong and other South-east Asian regions.

Materials and methods

Virus culture. Foot-and-mouth disease virus was isolated from the epithelium surrounding an erupted vesicle from a pig displaying clinical symptoms of FMD. The tissue sample was used to infect baby hamster kidney (BHK-21) cells cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) foetal calf serum (FCS) as described previously [9]. The Agriculture, Fisheries and Conservation Department of the Hong Kong SAR, China, generously provided the virus.

RNA extraction, RT-PCR, and fragment purification. Infected cells were lysed by repeated freeze-thaw. Cell debris was removed by centrifugation for 10 min at 4000 rpm. Total RNA was extracted from the supernatant by RNAgents Total RNA Isolation System (Promega, Madison, USA). Extracted RNA was reverse transcribed using Superscript II RT (Invitrogen, Carlsbad, CA, USA) and the virus RNA-specific primer R1 (5′-AAG AGA CTG GAG AGC GAG TCG GAG ATC TTC-3′). The first strand cDNA was subjected to PCR amplification using a pair of specific primers, comprising FVP1 (forward, 5′-GCT GAC TAC GCG TAC ACC GCG TCC-3′) and R1, to amplify a 1.2 kb fragment containing the VP1 sequence flanked on either side by 200–400 bp. A 50 μl PCR mixture contained 2 mM $MgCl_2$, 0.2 mM dNTP mix, 0.1 μM of each primer, 2.5 U Ex Taq DNA polymerase (Takara Bio, Shiga, Japan), 5 μl of 10× buffer, and 3 μl cDNA template. The PCR conditions were: 94 °C, 5 min (1 cycle); 94 °C, 30 s; 65 °C, 30 s; 72 °C, 90 s (30 cycles); 72 °C, 10 min; and 4 °C, infinity (1 cycle). The PCR products were separated on a 1% agarose gel by electrophoresis and the target band was recovered from the gel with the DNA Fragment Quick Purification/Recovery Kit (DingGuo Biotechnology, Beijing, China).

Cloning and sequencing. The purified fragment was cloned directly into a pGEM T Easy vector (Promega, Madison, USA). *Escherichia coli* DH5α was transformed with the vector using heat shock. Positive clones were sequenced using M13+/− universal primers (Shanghai Sangon Biologic Engineering Technology and Service, Shanghai, China).

Nucleotide sequence alignment and comparison. Reference FMDV sequences were obtained from the National Center for Biotechnology Information (NCBI) website (www.ncbi.nlm.nih.gov). Sequence alignments were made using DNAMAN Sequence Analysis Package Version 4.0 (Lynnon, Quebec, Canada). Sequence comparisons were made using the BLAST and BLAST 2 sequences software available from the NCBI website using default search parameters.

Results

Nucleotide sequence of HKN/2002 VP1

The nucleotide and the translated amino acid sequences of HKN/2002 VP1 are shown in Fig. 1. The VP1 coding region contained 639 nucleotides, encoding a 213 amino acid residue protein.

Nucleotide sequence comparison

A nucleotide sequence comparison conducted using the BLAST program with default search parameters indicated that HKN/2002 VP1 had the greatest sequence similarity to FMDV isolates of serotype O. A comparison of the nucleotide and amino acid sequences with the 18 most similar sequences identified using the BLAST program is shown in Table 1. Compared with these 18 isolates, the nucleotide identity ranged from 92.96% to 95.77%. The amino acid identity ranged from 94.37% to 96.71%. It is highly likely from an analysis of the VP1 coding region and its product that HKN/2002 belongs to serotype O.

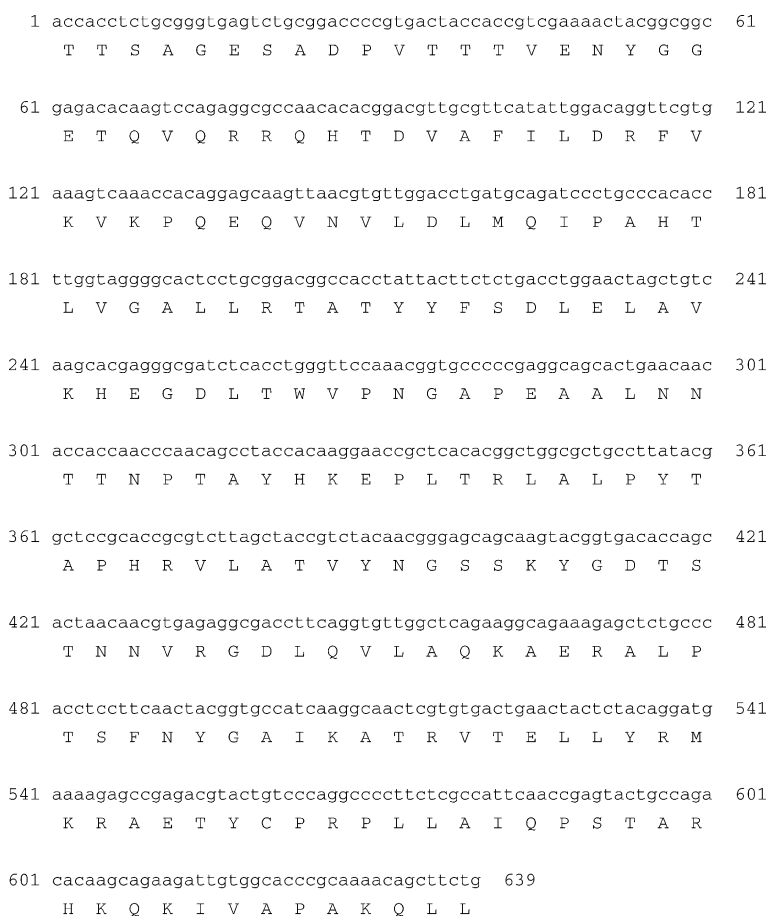

Fig. 1. The nucleotide and the translated amino acid sequences of HKN/2002 VP1. Amino acid residues are indicated below the nucleotide sequence by their single-letter codes.

Table 1
Similarity between HKN/2002 VP1 and the most closely related isolates obtained from sequence comparison

GenBank Accession No.	Serotype	Strain	Location	Nucleotide sequence similarity (%)	Amino acid sequence similarity	
					Identical residues (%)	Conserved residues (%)
12054125	O	O/HKN/1/99	Hong Kong	612/639 (95.77)	206/213 (96.71)	208/213 (97.65)
15211966	O	O/HKN/10/99	Hong Kong	601/639 (94.05)	201/213 (94.37)	202/213 (94.84)
12054119	O	O/HKN/7/96	Hong Kong	601/639 (94.05)	201/213 (94.37)	205/213 (96.24)
12054117	O	O/HKN/12/91	Hong Kong	602/639 (94.21)	205/213 (96.24)	209/213 (98.12)
12054123	O	O/HKN/20/96	Hong Kong	601/639 (94.05)	204/213 (95.77)	207/213 (97.18)
7329947	O	Taoyuan-113	Taiwan	597/639 (93.43)	204/213 (95.77)	209/213 (98.12)
7329961	O	Miaoli-165	Taiwan	596/639 (92.27)	205/213 (96.24)	210/213 (98.59)
5921457	O	Chu-pei	Taiwan	595/639 (93.11)	203/213 (95.31)	208/213 (97.65)
7329963	O	Tainan-168	Taiwan	595/639 (93.11)	204/213 (95.77)	209/213 (98.12)
7329957	O	Kaohsiung-153	Taiwan	595/639 (93.11)	204/213 (95.77)	209/213 (98.12)
7329955	O	Taipei-150	Taiwan	595/639 (93.11)	204/213 (95.77)	209/213 (98.12)
7329951	O	Yunlin-136	Taiwan	595/639 (93.11)	204/213 (95.77)	209/213 (98.12)
5031481	O	Tao-YuanTW97	Taiwan	595/639 (93.11)	204/213 (95.77)	209/213 (98.12)
7329939	O	Nantow-089	Taiwan	594/639 (92.96)	203/213 (95.31)	209/213 (98.12)
7329931	O	Tainan-041	Taiwan	594/639 (92.96)	203/213 (95.31)	208/213 (97.65)
7329929	O	Taoyuan-018	Taiwan	594/639 (92.96)	203/213 (95.31)	208/213 (97.65)
12054127	O	O/PHI/7/96	Philippines	594/639 (92.96)	204/213 (95.77)	208/213 (97.65)
4103946	O	TLTaiwan97	Taiwan	594/639 (92.96)	204/213 (95.77)	208/213 (97.65)

The HKN/2002 VP1 nucleotide and derived amino acid sequences were used to search the NCBI GenBank database using the BLAST program with default search parameters.

Sequence similarity tree

The complete VP1 nucleotide sequences of selected FMDV-O serotypes isolated from outbreaks in China (Mainland, Hong Kong, and Taiwan) and other countries bordering Mainland China (i.e., Bangladesh, Bhutan, Laos, Mongolia, Myanmar, Nepal, Pakistan, Tajikistan, and Vietnam) were used to construct a FMDV-O VP1-based sequence similarity tree (Fig. 2). From this analysis, HKN/2002 shared the greatest similarity at the nucleotide level with a group of Hong Kong FMDV-O viruses isolated from localised outbreaks during 1991–1999 and with isolates from a major FMD outbreak in Taiwan during 1997. Among the group of isolates examined, HKN/2002 was less similar at the nucleotide level to a disparate group of strains from other parts of western and South-east Asia.

Immunogenicity of HKN/2002

The major immunogenic sequence (βG–βH loop), formed from residues 135–160 in most FMDV serotypes, was obtained from a typical example of each of the seven major serotypes and compared with FMDV-O1/Manisa/Turkey/69 strain, a major component of FMDV-O vaccines (Table 2). The βG–βH loop domain of HKN/2002 was very similar to the O1/Manisa/Turkey/69 sequence. Thus, it is likely that a host immune response generated against O1/Manisa/Turkey/69 will afford some degree of protection against HKN/2002.

Origin of HKN/2002

The origin of FMDV HKN/2002 can be inferred only from indirect evidence. An analysis of pork products imported into Hong Kong from various countries is

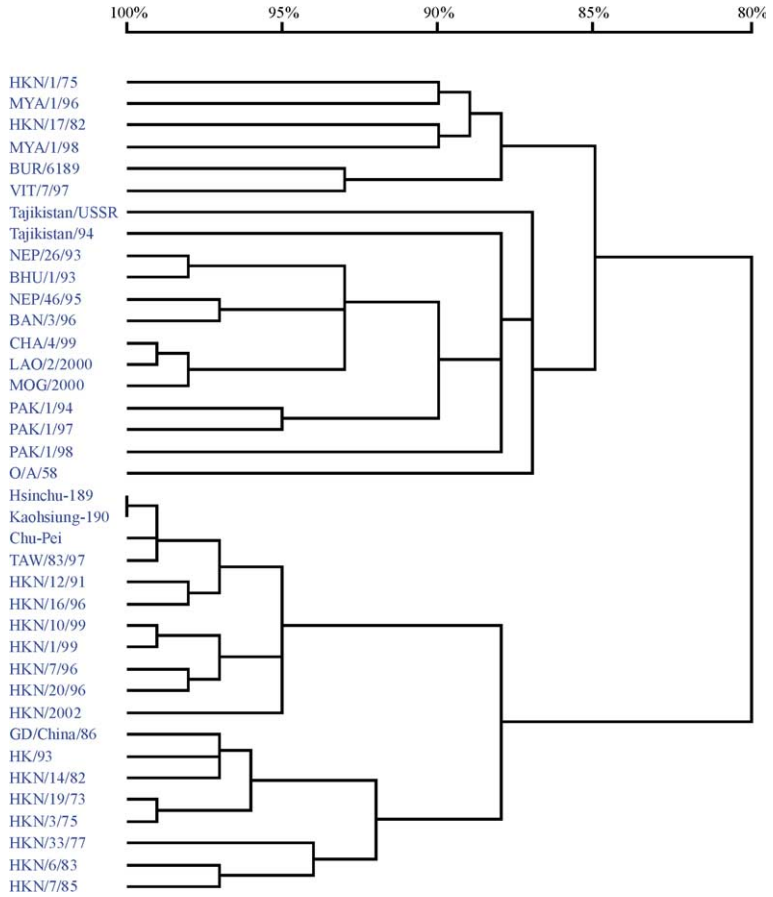

Fig. 2. Nucleotide sequence similarity tree based on a comparison of complete 1D (VP1) genes of selected FMDV-O isolates. The tree was constructed based on multiple sequence alignments made with Fast Alignment [10] using DNAMAN (4.0). The location of each FMDV isolate used in this table is abbreviated as follows: BAN, Bangladesh; BHU, Bhutan; CHA, China; HKN/HK, Hong Kong; LAO, Laos; MOG, Mongolia; MYA/BUR, Myanmar; NEP, Nepal; PAK, Pakistan; TAW, Taiwan; and VIT, Vietnam. The isolates can also be identified by their GenBank Accession Nos. (as listed in the figure in descending order): AJ294914, AJ303520, AJ294918, AJ303521, AJ294905, AJ296328, AJ004663, AJ004676, AJ303522, AJ303484, AJ303523, AJ303483, AJ318833, AJ318844, AJ318847, AJ303525, AJ303526, AJ318848, AJ131469, AF095884, AF095885, AF026168, AJ296322, AJ294921, AJ294923, AJ318836, AJ294925, AJ294922, AJ294924, AF525458, AJ131468, AJ131470, AJ294917, AJ294913, AJ294915, AJ294916, AJ294919, and AJ294920.

Table 2
Comparison of the major VP1 immunogenic region between FMDV serotypes

Isolate	GenBank Accession No.	Serotype	βG–βH loop protein sequence[a]	Amino acid similarity with respect to O1/Manisa/Turkey/69	
				Identical (%)	Conserved (%)
01/Manisa	6562397	O	KYGDGTVANV**RGD**LQVLAQKAARALP	26/26 (100)	26/26 (100)
HKN/2002	22094647	O	KYGDTSTNNV**RGD**LQVLAQKAERALP	21/26 (80)	22/26 (83)
63/72	1743240	Asia-1	TYGTQPTR**RGD**LAVLAQRVSNRLP	13/26 (50)	15/26 (58)
Buf2/28/1	2909840	SAT-1	KYKPAGTAPRDNI**RGD**LAVLAQRIAGETHIP	14/31 (45)	17/31 (55)
KEN/3/57	21389703	SAT-2	EYTKTVTAI**RGD**REVLAQKYSSAKHSLP	14/29 (48)	18/29 (62)
KNP/9/96/P	3414734	SAT-3	KYSKTQHVAPR**RGD**LAVLQQRVENETTRCRP	13/31 (42)	14/31 (45)
A Arg/68	15130854	A	KYTVSGSGR**RGD**MGSLAARVAKQLP	12/29 (41)	15/29 (52)
MARLS	10445392	C	TYTASA**RGD**SAHLTTTHARHLP	10/26 (38)	11/26 (42)

[a] Sequence based on amino acid residues 135–160 of O1/Manisa/Turkey/69. The highly conserved RGD receptor binding sequence is indicated in all serotypes by bold type.

Table 3
Origin and quantity (tonnes) of pork products imported into Hong Kong (1999–2001)

Origin	1999	2000	2001
PRC	37,730	42,440	53,650
Taiwan	50	3	4
Other countries[a]	95,520	118,237	118,036
Total imports	133,300	160,680	171,690

[a] Brazil, Netherlands, Canada, Germany, Denmark, and Greenland.

Table 4
Comparison of FMD in Hong Kong and Taiwan (1996–2001)

Year	Hong Kong		Taiwan	
	Outbreaks	Cases	Outbreaks	Cases
1996	17	NR	0	0
1997	0	0	6156	1,012,000
1998	7	2610	6	185
1999	0	0	0	0
2000	13	1883	6	76
2001	NR	3782	1	3

Data from Office International des Épizooties.
NR, not reported.

presented in Table 3. The occurrence of FMD in Hong Kong and Taiwan between 1996 and 2001 is compared in Table 4.

Discussion

Vaccination against FMD is an acknowledged means of controlling outbreaks of disease. Emergency vaccination coupled with rapid culling was useful in limiting the spread of FMD in several countries, including Albania (1996), Korea (2000), South Africa (2000), Uruguay (2001), and the Netherlands (2001), and has been endorsed as an option in controlling outbreaks in the United Kingdom in the wake of the large outbreak in 2001 [2]. Due to the multiple serotypes of FMDV in circulation, identification of the serotype affecting any one region is required in order to select the most appropriate antigens for inclusion in a vaccine preparation. The most important immunogenic site of FMDV is the VP1 surface antigen encoded by the 1D region. From a genomic sequence analysis of the VP1 isolated during a minor disease outbreak in Hong Kong in February 2002, the HKN/2002 isolate grouped with other typical serotype O strains (Fig. 1, Table 1).

Most previous outbreaks of FMD in Hong Kong in recent years have been attributed to FMDV-O isolates, although serotypes A and Asia-1 have been detected in cattle in Hong Kong in the 1970s (Dr. Leslie Sims, personal communication). This suggests that HKN/2002 represents a typical incursion of FMDV derived from either imported pigs or an isolate endemic to Hong Kong. The recommended FMD vaccine currently in use in Hong Kong includes antigens derived from FMDV-O1/Manisa/Turkey/69 and O-3039. A comparison of the major antigenic region of VP1 from HKN/2002 with that of O1/Manisa/Turkey/69 (Table 2) indicates a high degree of sequence similarity at the amino acid level (80% identical, 83% conserved). In contrast, the similarity of the comparable region in the other major serotypes to O1/Manisa/Turkey/69 is much lower. This indicates that HKN/2002 is likely to be well controlled by the current FMD management strategies comprising vaccination and controlled slaughter adopted in Hong Kong. However, it is often difficult to ascertain the likely degree of cross-protection provided by a vaccine based on similarities in gene sequence. A more thorough method would be to perform cross-protection studies using field isolates in experimentally inoculated animals.

From a modest phylogenetic analysis comparing 38 complete FMDV VP1 sequences from western and South-east Asia, three distinct groups of FMDV can be identified. The first group comprises isolates exclusively from Hong Kong (1973–2002) and Taiwan (1997). The second group comprises isolates mainly from central

Asia. The second group also includes isolates from Mongolia and Laos. The third group comprises a wide range of isolates mainly from South-east Asia (Myanmar, Vietnam, and Hong Kong). The third group is more closely related to the second group than to the first (Fig. 2). HKN/2002 appears to be most closely related to other FMDV isolates found in Hong Kong between 1991 and 1999, e.g., HKN/12/91, HKN/7/96, HKN/16/96, HKN/20/96, HKN/1/99, and HKN/10/99, with which it shares about 94–95% sequence similarity at the nucleotide level (Table 1, Fig. 2). This indicates that HKN/2002 shares a common ancestor with FMDV isolates occurring previously in Hong Kong, supporting the hypothesis that FMDV is present in an endemic low level that occasionally erupts into a localised epidemic. Hong Kong has a sizeable domestic pig-rearing industry, raising over 400,000 animals per year, where mandatory vaccination is not practised. It may be possible for FMDV to persist in a pig population under such circumstances, especially on fomites. The presence of asymptomatic carriers of FMDV exacerbates this problem. The Hong Kong pig-rearing industry is largely confined to the rural New Territories region bordering Mainland China. This region is also home to a small population (~700–800, 2002 estimate) of feral Asian water buffalo (*Bubalus bubalis*) and South China brown cattle that may act as a reservoir of FMDV. Domestic cattle can shed viable FMDV particles for 6–24 months whilst African buffalo (*Syncerus caffer*) can become lifelong carriers. However, the susceptibility of feral buffalo and cattle to pig-adapted strains of FMDV in Hong Kong is unknown. Pigs do not ordinarily become carriers following recovery from FMDV infection.

The HKN/2002 VP1 nucleotide sequence is as similar to FMDV isolates identified in Taiwan in 1997 as it is to other Hong Kong isolates from 1991, 1996, and 1999, e.g., Kaohsiung-190, Hsinchu-189, and Chu-Pei (Table 1, Fig. 2). The sequences of VP1 nucleotide and protein of HKN/2002 are most similar to that of a previous Hong Kong isolate—HKN/1/99. The rate at which the FMDV genome mutates has been estimated at about 1% per year [2] and so it is possible that HKN/2002 is a direct descendent of HKN/1/99, or at least shares a relatively recent common ancestor. The degree of divergence between the HKN/2002 and Taiwan 1997 VP1 nucleotide sequences (~3–4%) also supports the idea that these isolates also share a common ancestor. Consequently, it is possible that the large FMD outbreak in pigs in Taiwan in 1997 was a contributory factor in the occurrence of the disease in Hong Kong from 1997 to 2002. However, live pigs are not imported from Taiwan and the import of pork products is negligible compared with other markets (Table 3). In addition, there is no correlation between the number and severity of FMD outbreaks in Hong Kong and Taiwan (Table 4). Likewise, the origin of the Taiwan 1997 FMD outbreak could be attributed to carriage of the virus causing the 1996 Hong Kong outbreak on fomites in the opposite direction, which is supported by the VP1 nucleotide similarity between, for example, HKN/20/96 and Taoyuan-113 (Table 1) and the fact that a Hong Kong 1994 strain was identified as the causative agent of FMD outbreaks in Taiwan in 2000 and 2001 and the Philippines in 2000 [11]. The importance of fomites as a factor in sustaining FMD was made clear in an analysis of the 2001 UK outbreak [2]. FMDV can survive outside its host for periods up to 400 days [2]. While wind-borne carriage of FMDV over water has been reported over 250 km from a focus of infection [2], the distance between Hong Kong and Taiwan, at least 600 km, makes this possibility remote. Further genetic analysis is required to more closely correlate the relationship between Hong Kong and Taiwan isolates of FMDV, in which case wind-borne carriage of FMDV over water to a distance in excess of 600 km may be demonstrated. The persistence of FMD in Hong Kong has multiple and complex contributing factors that encompass inter-relations between host species, asymptomatic carriers, native fauna, fomites, climate and weather patterns, and the direction and frequency of animal and human transport.

This is the first report of the nucleotide and amino acid sequences of the VP1 gene and structural protein. The finding that HKN/2002 groups most closely with other serotype O isolates in a phylogenetic analysis indicates that it should be well controlled in existing serotype O vaccines. Pig farming brings considerable economic benefits to many small farmers in rural Hong Kong. The source of the apparently persistent infection should be identified in order to limit economic losses in future. The continued use of vaccination as an element of control strategies is recommended.

Acknowledgments

The authors thank Leslie Sims, Trevor Ellis, Pamela Li Chui Har, David Burrows, and C.C. Chan of the Agriculture, Fisheries and Conservation Department, Veterinary Laboratory Division, Tai Lung Experimental Station, Lin Tong Mei, Sheung Shui, Hong Kong SAR, China, for providing virus samples and technical assistance. This work was supported by Grant G1999011904 from the Special Funds for Major State Basic Research of China to J. Chen. The X. Chen was supported by Jun-Zheng Foundation in PKU.

References

[1] Anon., Manual of Standards for Diagnostic Tests and Vaccines, fourth ed., Office International des Épizooties, Paris, 2000.
[2] Anon., Infectious Diseases in Livestock: Policy Document 15/02, The Royal Society, London, 2002.

[3] R.A. Collins, L.S. Ko, K.Y. Fung, J. Xing, L.T. Lau, A.C.H. Yu, A method to detect major serotypes of foot-and-mouth disease virus, Biochem. Biophys. Res. Commun. 297 (2002) 267–274.
[4] F. Brown, N. Benkirane, D. Limal, H. Halimi, J.F. Newman, M.H. Van Regenmortel, J.P. Briand, S. Muller, Delineation of a neutralizing subregion within the immunodominant epitope (GH loop) of foot-and-mouth disease virus VP1 which does not contain the RGD motif, Vaccine 18 (1999) 50–56.
[5] G. Fox, N.R. Parry, P.V. Barnett, B. McGinn, D.J. Rowlands, F. Brown, The cell attachment site on foot-and-mouth disease virus includes the amino acid sequence RGD (arginine–glycine–aspartic acid), J. Gen. Virol. 70 (1989) 625–637.
[6] M.A. Martinez, J. Dopazo, J. Hernandez, M.G. Mateu, F. Sobrino, E. Domingo, N.J. Knowles, Evolution of the capsid protein genes of foot-and-mouth disease virus: antigenic variation without accumulation of amino acid substitutions over six decades, J. Virol. 66 (1992) 3557–3565.
[7] A. Villaverde, M.A. Martinez, F. Sobrino, J. Dopazo, A. Moya, E. Domingo, Fixation of mutations at the VP1 gene of foot-and-mouth disease virus, can quasispecies define a transient molecular clock?, Gene 103 (1991) 147–153.
[8] B. Pattnaik, R. Venkataramanan, C. Tosh, A. Sanyal, D. Hemadri, A.R. Samuel, N.J. Knowles, R.P. Kitching, Genetic heterogeneity of Indian field isolates of foot-and-mouth disease virus serotype O as revealed by partial sequencing of 1D gene, Virus Res. 55 (1998) 115–127.
[9] S. Curry, C.C. Abrams, E. Fry, J.C. Crowther, G.J. Belsham, D.I. Stuart, A.M. King, Viral RNA modulates the acid sensitivity of foot-and-mouth disease virus capsids, J. Virol. 69 (1995) 430–438.
[10] D.G. Higgins, P.M. Sharp, Fast and sensitive multiple sequence alignments on a microcomputer, Comput. Appl. Biosci. 5 (1989) 151–153.
[11] N.J. Knowles, Virus strain identification for the years 2000–2001: report for the OIE/FAO World Reference Laboratory for foot-and-mouth disease, Institute for Animal Health, Pirbright, 2001.