Epidemiology, Prevention and Control of Hand, Foot, and Mouth Disease in Hong Kong

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A Thesis Submitted in Fulfilment
of the Requirements for the Degree of
Doctor of Medicine

January 2013

Declaration of Originality

The work in this thesis is completely original. There is no funding source for this project and the source of data has been retrieved from the Department of Health with formal approval. I obtained advice on the process of research from Dr. SK Chuang, Consultant Community Medicine from the Centre for Health Protection.

I was responsible for the initial conception, study design, retrieval and assembly of raw data, critical analysis and interpretation of the study results, writing of the drafts of all original papers from which this thesis was composed, and writing of all parts of this thesis.

I would like to thank statisticians, Mr. Simon Wong and Mr. Steven Tip contributed to refining the statistical methodology and data analysis, and critical analysis of result interpretation. I would like to express my gratitude to my seniors, Dr. Thomas Tsang, Dr. SK Chuang and Dr. Christine Wong for their support of my thesis and contribution to intellectual input and critically revising the manuscripts.

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Acknowledgements

This thesis has accumulated my 10 years of experience in management of infectious diseases outbreaks and about three years work in research in this particular topic. I wish to express my sincere gratitude to my seniors, Dr. Thomas Tsang, Controller, Centre for Health Protection; Dr. SK Chuang, Consultant Community Medicine (Communicable Disease) and Dr. Christine Wong, Principal Medical Officer for their continuous support and invaluable advice in critically revising the manuscripts. My thanks also go to Professor Sian Griffiths, Director of the Jockey Club School of Public Health and the Primary Care, CUHK, who offered tremendous support for the submission of this thesis.

I would like to thank all medical, nursing and research staff in Epidemiology Section, Surveillance and Epidemiology Branch, Centre for Health Protection, for their work in investigating outbreaks of HFMD. I would like to express my gratitude to staff in Public Health Laboratory Centre for doing the laboratory tests for EV71 and other enteroviruses. I would like to thank all doctors participating in the sentinel surveillance system to monitor the trend of HFMD and the Hong Kong Observatory in providing the meteorological data.

Last but not the least, my heartily appreciations towards statisticians, Mr. Simon Wong and Mr. Steven Yip who gave their professional advice in refining the statistical methodology and data analysis, and interpretation of the statistical results.

Abbreviations

Cox	Coxsackievirus
DH	Department of Heath
EV71	Enterovirus 71
GOPC	General out-patient clinics
GP	General practitioners
HFMD	Hand, foot, and mouth disease
PPV	Positive predictive value
R_0	Reproduction number
SARS	Severe Acute Respiratory Syndrome
SD	Standard deviation
SSS	Sentinel Surveillance System
WHO	World Health Organization

Caption for Tables

Caption for Figures

Précis of the Thesis

Background

Hand, foot, and mouth disease (HFMD), in particular those associated with enterovirus 71 (EV71), has caused large outbreaks in the Western Pacific and Southeast Asian countries in the past three decades. There is currently no effective chemoprophylaxis or vaccination for HFMD or EV71 infection. Public health strategies rest on good understanding of the epidemiological features of HFMD. The present series of studies examined the epidemiological characteristics of HFMD in Hong Kong, with a view to better understand the disease epidemiology so as to guide public health actions.

Methods and results of individual studies

Study (1) — characterizing the changing epidemiological features identified from various surveillance systems for HFMD

The trend of HFMD activities from 2001-2009 was analyzed using the sentinel surveillance statistics and HFMD outbreaks. The type of institutions reporting HFMD over time, incidences of outbreaks in 18 districts, and age and sex distribution of affected persons of HFMD outbreaks were analyzed. The clinical presentation, hospitalization rate, complication rate and case fatality of outbreaks were examined. The circulating enterovirues each year were determined by laboratory surveillance findings from 2001-2009 and test for morbidity caused by EV71. Seasonal peak was detected from warmer months of May through July but a smaller winter peak was found from October to December since 2006. An increasing trend of more older

children aged above 5 years were infected, from 25.4% in 2001 to 33.0% in 2009 (p=0.01, Mantel-Haenszel Chi-square test). Laboratory surveillance detected a cyclical high activity of EV71 in every 3 to 4 years, which was associated with a higher average hospitalization rate among patients of the HFMD outbreaks reported in corresponding year, although it was only marginally significant (p=0.09, linear regression test).

Study (2) — analyzing the characteristics of EV71 epidemic in 2008

All EV71 cases diagnosed by PHLC from 1998-2008 were analyzed. The complication and case fatality rates, percentage requiring hospitalization, median duration of hospital care, and the likelihood of being associated with an HMFD outbreak in institution in 2008 were compared with the corresponding rates calculated from cases reported from 1998-2007. Phylogenetic tree was constructed by using the neighbour-joining method and the molecular epidemiology of EV71 detected in 2008 was compared with the past years' trends. Ninety-eight EV71 cases were reported in 2008, highest in the past decade. The annual incidence was 1.4/100 000 in general population, with highest incidence reported in children aged 0-4 years old (27.9/100 000). 11.2% had complications including meningitis or encephalitis (6.1%), pneumonia (3.1%), acute flaccid paralysis (1.0%), and shock (1.0%). There was only one fatal case (CFR: 1.0%) attributed to interstitial pneumonitis. 45.9% had concurrent HFMD outbreaks in their schools or institutions, and six schools required temporarily class suspension for 14 days. Both the complication rate and CFR were not significantly different from the corresponding rates of the past 10 years (p=0.12 and 1.00 respectively). Phylogenetic analysis found that most cases reported in 2008 were C4 strains, which were the predominant circulating strains in the past ten years.

Study (3) — examining the association between meteorological parameters and HFMD activity

The sentinel consultation rate of HFMD was tested for any association with the meteorological parameters obtained from the Hong Kong Observatory from 2000-2004. Different regression models were examined to find the best model for predicting HFMD consultation rates from 2005-2009. In multivariate regression analysis, model M2 (in which mean temperature, diurnal difference in temperature, relative humidity and wind speed were positively associated with HFMD) was found to have a higher R^2 (0.119) than M0 and M1 models with an R^2 of 0.079 and 0.062 respectively, indicating that HFMD consultation rates were better explained using meteorological parameters measured 2 weeks earlier. The predicted trend of HFMD consultation rates for 2005 to 2009 matched well with the observed one (Spearman's rank correlation coefficient=0.276, P=0.000). Sensitivity analysis showed that the estimated HFMD consultation rates were mostly affected by varying the relative humidity and least affected by wind speed.

Study (4) — determining the basic reproduction number of coxsackievirus A16 and enterovirus 71 using mathematical model

The basic reproduction numbers (R_0) of EV71 and CoxA16 from laboratory confirmed HFMD outbreaks reported to DH from 2004-2009 were determined using mathematical model. Thirty four outbreaks were analyzed, 27 due to CoxA16 and seven due to EV71. The median R_0 of EV71 was 5.48 with an inter-quartile range of 4.20–6.51 while median R_0 of CoxA16 was 2.50 with an inter-quartile range of 1.96–3.67. In the sensitivity analysis, R_0 of EV71 was significantly higher than that of CoxA16 in whole range of incubation periods, $p \leqq 0.025$. R_0 was not associated with outbreak setting, size of the institution or number of persons affected.

Study (5) — assessing the impact of SARS and pandemic influenza H1N1 on transmission of HFMD in Hong Kong

I compared the observed HFMD consultation rates and the projected rates, which were constructed using mathematical model, in defined periods of 2003 and 2009 during which territory-wide public health interventions (including school closure) against Severe Acute Respiratory Syndrome (SARS) and pandemic influenza H1N1 were implemented. There was a reduction of 57.2% (95% C.I.:53.0-60.7%) in observed HFMD consultation rates during SARS period in 2003 and a reduction of 26.7% (95% C.I.:19.5-32.7%) during pandemic influenza H1N1 period in 2009. In 2003, the projected rates were still lower than the observed rates beyond week 31 until almost the end of the year. On the contrary, in 2009, the observed HFMD consultation rates became comparable to that of the projected rates in August, before the end of the defined intervention period.

Conclusions

This thesis bridges the knowledge gaps regarding epidemiological characteristics of HFMD. The changing epidemiology of HFMD, including the cyclical high activity of EV71 warrants vigilant surveillance of its activity in order to guide preventive measures. I have demonstrated that climate parameters may help predict HFMD activity, which could assist in explaining the winter peak detected in recent years and issuing early warning in the future. The R_0 of EV71 and CoxA16 were first determined in the literature and I found that R_0 of EV71 was higher than R_0 of CoxA16. The reduction of transmission of HFMD during the SARS and H1N1 periods suggested that public health measures are effective in reducing the transmission of enteroviruses.

PART I :

LITERATURE REVIEW OF HFMD

Chapter 1. Current Understanding of Epidemiology of Hand, Foot, and Mouth Disease

1.1 Causative agents and virology

HFMD is caused by various serotypes of enteroviruses. *Enterovirus* is classified under the family Picornaviridae which contains 12 genera of non-enveloped, linear positive-sense, single-stranded RNA viruses. Other examples of Picornaviridae include *Aphthovirus*, *Cardiovirus* and *Hepatovirus*. Enteroviruses refer to a group of small RNA viruses with a diameter of 24-30nm comprising four species, namely Polioviruses (3 serotypes), Coxsackieviruses (group A: 23 serotypes, group B: 6 serotypes), Echoviruses (31 serotypes) and Enteroviruses type 68-71. The currently recognized species and types of Enterovirus are listed in Table 1.1 (Wong S.S. et al, 2010). The commonest cause for HFMD is CoxA16 while other types of enteroviruses have also been associated with this syndrome, such as coxasackievirus A4, A5, A9, A10, B2, B5 and EV71.

The genome of human EV71 has been intensively studied because of its association with past epidemics. The genome of EV71 is about 7.4 kb in size, which is flanked by 5k and 3k untranslated regions (Wong S.S. et al, 2010). The protein-coding region can be divided into three regions: P1 encodes for the structural proteins VP4, VP2, VP3, and VP1; P2 and P3 for the non-structural proteins 2A, 2B, 2C and 3A, 3B, 3C, 3D, respectively (Muir, 1998; McMinn, 2002). EV71 has three genotypes (A, B, and C) based on VP1 and VP4 gene sequences. Genotypes B and C are each further divided into five subtypes B1–B5 and C1–C5, respectively. VP1 carries most of the

type-specific neutralizing antibody epitopes. Mutations in VP1 have been associated with increased virulence in animal models (Chua BH, 2008).

Table 1.1 Enteroviral species and types currently recognized

Enterovirus species	**Types**
Human enterovirus A	Human Coxsackievirus A2–8, 10, 12, 14, 16. Human enterovirus 71, 76, 89–92.
Human enterovirus B	Human Coxsackievirus A9, B1–6. Human echovirus 1–9, 11–21, 24–27, 29–33. Human enterovirus 69, 73–75, 77–88, 93, 97, 98, 101, 106, 107.
Human enterovirus C	Human poliovirus 1–3. Human Coxsackievirus A1, 11, 13, 15, 17–22, 24. Human enterovirus 95, 96, 99, 102, 104, 105, 109.
Human enterovirus D	Human enterovirus 68, 70, 94.
Human rhinovirus A, B, C	
Porcine enterovirus B	
Bovine enterovirus	
Simian enterovirus A	

1.2 Clinical manifestations and management

Clinical presentation

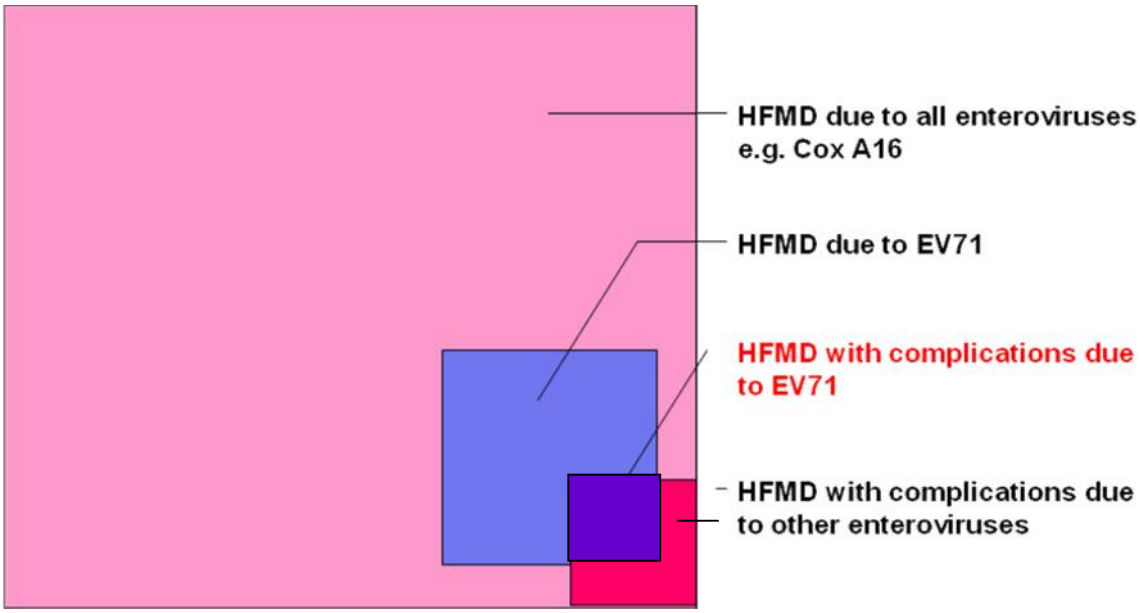
Enteroviral infections may be asymptomatic or present with HFMD or herpangina with or without manifestations of other clinical syndromes. The disease usually presents with rashes over fingers and palms, feet and other parts of the body such as buttocks and thighs. Vesicles can be found on the tongue and soft palate. The oral enanthem helps to distinguish HFMD from other causes of childhood exanthems, although cases without oral lesions have been reported. The clinical syndromes of HFMD caused by different enteroviruses such as CoxA4, A6, A16, B3, B5, echoviruses 11, 18, are essentially non-distinguishable. In most cases, the illnesses are self-limiting and the patients can recover completely in 5 to 7 days.

Although most patients present as mild illness, some patients may develop severe complications such as meningitis, encephalitis, acute flaccid paralysis, myocarditis, pulmonary edema, or even death (Ho M. et al, 1999; AbuBakar et al, 1999a; Shekhar et al 2005; Jan et al, 2000; Gilbert et al, 1988; Centres for Disease Control and Prevention, United States, 1998; Chong et al, 2003; Shah et al, 2003; Ho M., 2000). Neurological syndromes included aseptic meningitis, Guillain-Barre syndrome, acute transverse myelitis, acute cerebellar ataxia, opso-myoclonus syndrome, benign intracranial hypertension, and afebrile convulsion (McMinn et al, 2001b; Wang J.W., Xue & Sun, 2009; Liao & Hung, 2001; Cho et al, 2010). In rare occasion, intrauterine infection of EV71 was reported and the pregnancy was complicated by stillbirth with virological evidence of brain and liver involvement (Chow et al, 2000). Another case report revealed perinatal infection by EV71 affecting a baby who presented with HFMD and aseptic meningitis and recovered without long term neurological sequelae (Nishikii et al, 2002). Co-infection of enteroviruses is possible. In Sarawak of

Malaysia, 10% of the patients were found to have co-infection of EV71 with other viruses (Ooi et al, 2007b). However, co-infection does not seem to cause more severe clinical diseases (Chan K.P. et al, 2003).

The relationship between HFMD and severe complications as well as the different causative agents is illustrated in Figure 1.1. Most HFMD due to enteroviruses (e.g. CoxA16) are mild (light pink area), including those caused by EV71 (light blue). However, some enteroviruses (deep pink), in particular EV71 (deep blue), would cause severe complications. Among all these types of infections, HFMD with complications due to EV71 are of most concern.

Figure 1.1. Relationship between HFMD and severe complications due to different enterovisures.



It should be noted however that many EV71 infections do not cause HFMD but present with severe illnesses. For example, the epidemic in Bulgaria in 1975 involving 705 cases including 68 fatalities were characterized by neurological symptoms instead

of HFMD (Shindarov et al, 1979). Similarly, the Hungary outbreak affecting 323 cases had only four cases presenting with HFMD (Nagy et al, 1982). Deibel et al reported nine cases of meningitis and one case of encephalitis due to EV71 but only one case had HFMD in the New York outbreak in 1972 (Deibel, Gross & Collins, 1975).

Pathogenesis

The mostly studied pathogenesis accounting for HFMD is that relating to EV71. Three human cellular receptors of EV71 have been identified, namely human P-selectin glycoprotein ligand-1 (CD162), DC-SIGN [human dendritic cell-specific intracellular adhesion molecules (ICAM)-3 grabbing non-integrin, also known as CD209], and the scavenger receptor BII(SR-BII)(Lin Y.W. et al, 2009; Nishimura et al, 2009; Yamayoshi et al, 2009). The presence of CD162, a cell surface glycoprotein, on macrophages of the gastrointestinal tract has been suggested as the primary site of viral multiplication after infection while infection and activation of Langerhans cells in the skin account for the dermatological manifestations of HFMD (Nishimura et al, 2009). The systemic infection and predilection for involvement of central nervous system may be attributed to the ubiquity of EV71 receptors in different organs. Patients with encephalitis had inflammatory changes of oedema, vascular congestion, neuronal degeneration and even necrosis (Chan L.G. et al, 2000; Shekhar et al, 2005). For those who had suffered from brainstem encephalitis, their myocardium showed coagulative myocytolysis and myofibrillar degeneration (Fu et al, 2004; Shieh et al, 2001). This supports the postulation that the pathogenesis is of neurogenic cardiac damage instead of direct cardiac involvement (Wang J.W., Xue & Sun, 2009). Similar, pulmonary oedema has been suggested to be neurogenic origin from post-mortem studies (Lum et al, 1998; Cardosa et al, 1999; Huang et al, 1999; Wang et al, 1999; Ng

et al, 2001). Magnetic Resonance Imaging studies of children with EV71 brainstem encephalitis showed inflammation of grey matter of the spinal cord and the whole medulla oblongata (Huang C.C. et al, 1999; Lum et al, 1998; Wong K.T. et al, 2008; Hsueh, et al, 2000; Shen et al, 1999). Fulminant pulmonary oedema is probably preceded by and closely associated with central nervous system involvement.

Laboratory diagnosis

The diagnosis of HFMD mainly rests on clinical presentation but the exact viral causative agents can be identified by various laboratory tests. Vesicular fluid aspirated from skin lesion, nasopharyngeal swabs or throat swabs can be obtained from patients infected with enterovirus infections. Stool or rectal swabs can be considered for small children from whom taking nasopharyngeal swabs or throat swabs might be difficult. For those with central nervous system involvement, cerebrospinal fluid can be used for viral detection.

The commonest choice of rapid diagnostic test nowadays is reverse transcription-polymerase chain reaction of the RNA extracted from the aforementioned specimens, targeting towards the 5' undtranslated or VP1 region of the viral genome (Iturriza-Go'mara, Megson & Gray, 2006; Leitch et al, 2009; Nix, Oberste & Pallansch, 2006). Enteroviruses can be isolated using conventional cell culture or rapid shell viral culture. Immunostaining with specific monoclonal antibodies against VPi proteins can differentiate between EV71 from CA16. Serological diagnosis requires a fourfold rise in neutralizaing antibody titre with a minimum of 10 to 14 days apart. Immunoglobulin M antibody to EV71 has been detected but the test is not widely available (Tsao et al, 2002, Xu F. et al, 2010; Ju et al, 2010).

Clinical management

Most of the HFMD are self-liming and no specific antiviral treatments are available for EV71 or other etiologic agents causing HFMD. Supportive treatment remains the principle of management. It is important to ensure adequate fluid intake to prevent dehydration. Cold liquids are generally preferable over acidic substances which may cause discomfort. Intravenous hydration may be necessary if the patient has moderate to severe dehydration or if discomfort precludes oral intake. Fever may be treated with antipyretics while pain may be treated with standard doses of acetaminophen or ibuprofen. Direct analgesia may also be applied to the oral cavity via mouthwashes or sprays.

Pleconaril is an antiviral that integrates into the capsid of picornaviruses, including enteroviruses and rhinoviruses, and prevent the virus from attaching to cellular receptors and uncoating to release RNA into the cell. Unfortunately, pleconaril is not active against EV71. Pleconaril has been shown to provide significant therapeutic benefit in aseptic meningitis, acute flaccid paralysis and encephalitis due to many enterovirus serotypes (Rotbart, 2000; Pevear et al, 1999; Starlin et al, 2001; Rotbart & Webster, 2001; Rotbart, O'Connell & McKinlay, 1998). In a clinical trial of 36 patients, 28 patients (78%) including 12 of 16 with chronic enterovirus meningoencephalitis, were shown to have a clinical response temporally after treatment of pleconaril therapy (Rotbart & Webster, 2001). In the same study, nearly all patients whose virological responses (12 [92%] of 13), laboratory responses (14 [88%] of 16), and radiological responses (3 [60%] of 5) could be evaluated were judged to have responded favorably to a course of pleconaril treatment (Rotbart & Webster, 2001). Pleconaril is readily absorbed after oral use and it penetrates well into body fluids including the cerebrospinal fluid. Adverse effects were minimal and the

drug was generally well-tolerated. Other antiviral such as ribavirin and interferons have been studied (Li et al, 2008; Liu M.L. et al, 2005) and RNA interference approaches are being explored but their effectiveness are not conclusive (Sim et al, 2005; Wu Z. et al, 2009; Tan et al, 2007).

Maternal antibody appears to be protective against enteroviruses and transfusion of maternal plasma had been used in a case of neonatal disseminated echovirus infection (Jantausch et al, 1995). Intravenous immunoglobulin has been studied and widely used in Taiwan and Australia where large outbreaks have occurred (Cheng M.F., et al, 2008; Moschovi et al, 2007; McMinn et al, 2001b; Huang C.C., 2001; McKinney Jr., Katz & Wilfert, 1987). Immunoglobulin was assumed to be able to neutralize the virus and has non-specific anti-inflammatory actions (Ooi M.H. et al, 2007b; Wang S.M. et al, 1999). The presence of neutralizing antibodies enables it to suppress viral replication. Besides, through its anti-inflammatory activities, immunoglobulin may also limit organ damage and hence was suggested to be used in patients with complicated EV71 infection (Nolan et al, 2003; Wang S.M. et al, 1999; Frange et al, 2007; Wang S.M. et al, 2006). It has been demonstrated that patients who did receive immunoglobulin suggest a benefit from this treatment if given early (Ooi M.H. et al, 2009; Chang et al, 2004b). There was significant reductions in concentration of cytokines before and after treatment of immunoglobulin among patients with EV71 complicated with encephalitis but not among patients with mild illness (Simmonds & Welch, 2006; Mizuta et al, 2009; Van der Sanden et al, 2010; Kung et al, 2007; Chan Y.F. & Abu Baker, 2004). Although it is now widely used in Taiwan as national treatment guideline for severe EV71 infections, the benefits have not been demonstrated in any randomized controlled trial (Lin T.Y. et al, 2002; Chang et al, 2004b; Wang J.N. et al, 2006).

Apart from pleconaril, milrinone has been shown some efficacy in a few reports (Thomas & Janniger, 1993; Toida et al, 2003; Wang S.M. & Liu, 2009). Milrinone is a cyclic nucleotide phosphodiesterase inhibitor for treating congestive heart disease. By inhibiting phosphodiesterase milrinone acts by increasing the intracellular concentrations of cyclic AMP which in turn results in increased cardiac output and decreased pheripheral vascular resistance. The clinical benefits have been demonstrated in a historically controlled study in Taiwan that patients with pulmonary oedema due to EV71 and treated with milrinone were shown to have longer survival and better clinical outcome (Wang S.M. et al, 2005; Wang S.M. et al, 2006).

1.3 Geographical distribution and past epidemics

Global geographical distribution

HFMD is very commonly reported in various parts of the world, in particular among South-east Asian areas such as Taiwan, Malaysia, the Philippines, Singapore, South Korea, Mainland China and Hong Kong. Several waves of epidemic of HFMD have been documented in the past three decades (Please see Chapter 3). Although non-polio enteroviruses are distributed worldwide, the prevalence varies with time and place. The reason for the difference in geographical distribution is uncertain, but the association with human leukocyte antigen-A33, which is common in some Asian populations and is associated susceptibility to EV71 infection has been suggested (Chang et al, 2008). Other factors such as genetic predisposition, poor environmental hygiene, and micronutrient deficiencies require further studies to confirm the significance (Beck & Matthews, 2000; Beck, Williams-Toone & Levander, 2003; Cermelli et al, 2002; Mata, Urrutia & Lechtig, 1971; Le et al, 2004). The seasonality

of HFMD detected in different areas suggests that climate factors may have a role to play in determining the activities of HFMD (Ang et al, 2009; Chen K.T. et al, 2007; Podin et al, 2006). In countries with temperature climates, epidemics tend to occur in summer and autumn months while the infections are common throughout the year in tropical regions (Ang et al, 2009; Chen K.T. et al, 2007; Druyts-Voets, 1997; Ho M., 2000; Podin et al, 2006; Sasidharan et al, 2005). Associations between climate factors and various infections, particularly influenza, have been studied (Lowen et al, 2007; Zuk, Rakowsk & Radomski, 2009a; du Prel et al, 2009; Chan P.K. et al, 2009). Chan P.K. et al has demonstrated that cold and humid conditions are associated with a higher activity of both influenza A and B in Hong Kong (Chan P.K. et al, 2009). Zuk has demonstrated that spread of influenza virus depended on various temperature and relative air humidity levels (Zuk, Rakowsk & Radomski, 2009a). On the other hand, there is only limited literature identified in this aspect for HFMD. In a Japanese study examining the relationship of weather conditions and HFMD and herpangina cases detected at sentinel paediatric clinics, it was shown that higher air temperature and humidity or vapor pressure, and lower precipitation and duration of sunshine increased the incidence of HFMD or herpangina (Urashima, Shindo & Okabe, 2003).

Epidemics in the past

There were three waves of HFMD epidemics in the world, one in each decade between 1970 to 2000, with the most well known epidemic in Southeast Asia areas in late 1990s (Bible et al, 2007). Interestingly, another wave of epidemic occurred in late 2000s, ten years apart from the last epidemic in the Region. Major outbreaks of HFMD in the world are summarized in Table 3.1. Table 3.2 shows the summary of predominant EV71 genotypes in the Asia Pacific Region.

Early report of outbreaks before 1980s

Schmidt et al, reported the first outbreak of EV71 in California of the United States in 1969 affecting 20 cases of meningitis and encephalitis (Schmidt, Lennette & Ho, 1974). Later two larger outbreaks were reported in Bulgaria in 1975 and Hungary in 1978 respectively (Shindarov et al, 1979; Nagy et al, 1982). From May to September, 1975, Bulgaria recorded 705 cases of febrile illness attributable to EV71, of which 77.3% presented with aseptic meningitis while 21.1% presented with acute flaccid paralysis (Shindarov et al, 1979). EV71 was isolated from 25.3% of the clinically diagnosed cases and from 100% of fatal cases. Again in summer (May to September) of Hungary, a total of 826 cases of aseptic meningitis and 724 cases of encephalitis were reported, with the latter group including cases of cerebellar ataxia and acute flaccid paralysis (Nagy et al, 1982, Fujimoto et al, 2008). EV71 was confirmed in 323 cases. In contrast to Bulgarian epidemic, four cases of HFMD associated with EV71 infection were documented in Hungary outbreak. These early reports of enterovirus outbreaks predominantly involved neurological complications associated with EV71. The first large HFMD outbreak was reported in Japan in 1975 affecting 3296 cases, which was also caused by EV71 (Tagaya and Tachibana, 1975). Three years later (in 1978) in Japan, another outbreaks occurred affecting 36301 cases Hagiwara, Tagaya & Yoneyama, 1978; Hagiawara et al, 1984).

In 1980s, outbreaks due to EV71 were also reported in various parts of the world including Australia and the United States, with significant numbers of aseptic meningitis, brain stem encephalitis and death (Gilbert et al, 1988; Alexander et al, 1994). A small EV71 outbreak which affected several cases of acute flaccid paralysis was identified in Hong Kong in 1985 while a small HFMD outbreak with complications of aseptic meningitis due to EV71 was reported in Singapore in 1987

(Samuda et al, 1987; Doraisingham, 1987). The first report of EV71 activity in China was the HFMD epidemic in Hubei Province in 1987, although no cases of aseptic meningitis or acute flaccid paralysis were identified (Zheng et al, 1995).

Epidemics in Asia in late 1990s

Large outbreaks due to enterovirus 71 have occurred from 1997 to 2000 in Asian countries including Malaysia, Taiwan, Singapore, and Japan (AbuBakar, 1999a; Ho M. et al, 1999; Fujimoto et al, 2002; Shah et al, 2003). The alarming feature was the appearance of a syndrome of rapidly fatal neurologic pulmonary oedema associated with severe brainstem encephalitis (Chang, Huang & Lin, 1998; Lum et al, 1998). From April through June 1997, 29 previously healthy children aged <6 years in Sarawak, Malaysia, died of rapidly progressive cardiorespiratory failure during an outbreak of HFMD caused primarily by EV71 (Chan L.G. et al, 2000). The largest HFMD outbreak occurred in 1998 in Taiwan affecting over 129000 cases of HFMD or herpangina, which were estimated to account for only less than 10% of the true figure (Chang L.Y. et al, 2002). That outbreak resulted in 405 severe cases with complications such as encephalitis, aseptic meningitis, pulmonary edema or haemorrhage acute flaccid paralysis, and myocarditis (Ho M. et al, 1999). This outbreak eventually resulted in 78 deaths mostly (92%) associated with EV71. Radiological, histopathological, virological and serological studies all showed the cases presenting with acute pulmonary oedema and other neurological disease in Taiwan were due to brainstem encephalitis resulting from acute EV71 infection (Liu C.C. et al, 2000; Liao & Hung, 2001; Huang C.C. et al, 1999; Hsueh et al, 2000; Chang et al, 1999b; Yan et al, 2000). Risk factors for acquisition of EV71 infection in Taiwan were identified as 1) having an older sibling with positive EV71 serology, 2) age between 6 months and 3 years of age, 3) number of children in the family, and 4)

history of contact with a case of HFMD or herangina (Chang, 2000).

These outbreaks have far reaching public health implication in terms of awareness of the disease and enhanced surveillance. For example, Malaysia has set up the sentinel surveillance system in March 1998 to regularly monitor HFMD activity after the large outbreak occurred in Sarawak resulting in paediatric deaths due to EV71 associated encephalitis and cardiac failure in 1997 (Podin et al, 2006). Similarly, in Hong Kong, HFMD was also added as one of the disease monitored by the sentinel surveillance system of general practitioners in mid-1998. In Taiwan, while recognizing the significant mortality and morbidity of the disease in 1998 epidemic, the Ministry of Health established a new system, in addition to the pre-existing sentinel system, for detecting severe and fatal cases of enterovirus infections in the same year (Wu T.N. et al, 1999).

Recent epidemics in late 2000s

From 2008 to 2010, another wave of large HFMD epidemic occurred in the Region including Mainland China, Taiwan, Singapore, and Hong Kong, also associated with an increased activity of EV71 (Ding et al, 2009; Huang S.W. et al, 2009; Singapore Ministry of Health, 2008a; Ma et al, 2010a). The EV71 outbreak in Auhui Province, China has triggered much public alarm in 2008. The case fatality rate was as high as 0.3% in the outbreak occurring in Fuyang City of Anhui Province compared with the overall case fatality rate of about 0.003% reported in China in 2008 (Chinese Center for Disease Control and Prevention & WHO, 2008). In Hong Kong, there were a total of 163 HFMD outbreaks occurring in institutions in 2008, affecting 967 persons, including eight severe infections and one fatal case (Tsui & Ma, 2008; Lau A. et al, 2009). HFMD activity in Singapore in 2008 was also higher than it was previously

reported (Singapore Ministry of Health, 2008b). The recurrence of epidemic in the Region in 2009 has brought attention of the WHO to urge for vigilance against the disease (WHO, 2009b).

These epidemics brought much public health attention and implications. In 2009, Korea has added HFMD as one of their notifiable disease (Kim, 2010). In the same year in Hong Kong, EV71 was added as a statutory notifiable disease and doctors in Hong Kong have to report these infections by law to the DH (Ma & Chan, 2009). Besides, health authorizes have adopted more stringent public health control in halting the disease transmission, probably after the lesson learnt from the SARS epidemic. For example, the Singapore Ministry of Health has ordered 35 child care centre, preschools and playgroups to close mandatorily while 95 closed voluntarily during the 2008 epidemic in Singapore (Ministry of Health, Singapore, 2008a). In Hong Kong, eight kindergartens / child care centres and one primary school required class suspension in the 2010 epidemic (Centre for Health Protection, Hong Kong, 2010).

HFMD in other countries outside Asia appear to be less active in 2000s. Two small community outbreaks of neurological EV71 disease, without HFMD, occurred in 2003 and 2005 in Denver of USA, affecting 6 children aged 4 weeks to 9 years and one child died (Perez-Velez et al, 2007). A study in the United Kingdom identified 32 sporadic cases of EV71 infection between 1998 and 2006, presenting primarily as neurological disease, HFMD, or both (Bible et al, 2008). In the Netherlands, 58 people were admitted to hospital with EV71-associated fever, gastrointestinal symptoms, and central nervous system infections in 2007, after 21 years of low endemicity (Van der Sanden et al, 2009).

Cyclical activity of enterovirus infection

The reasons why epidemics occurred in the observed patterns are multiple. Accumulation of susceptible population probably account for the cyclical activity of HFMD. Epidemics of HFMD occurred once every two to five years. This is compatible to the fact that the most susceptible population is under 5 years old. It has been suggested that the quiescence between epidemics is due to the development of population immunity that occurs during the high-infection-rate epidemic (Centers for Disease Control and Prevention, United States, 2000). During the years of the quiescence of the virus, the accumulation of a susceptible population, especially in younger cohorts, probably contribute to the next epidemic. In addition, emergence of a new genotype may also lead to unexpected outbreaks. Epidemics in Taiwan in 1998 and 2000 have been attributed to changes in prevailing strains of C2 and B4 respectively (Wang J.R. et al, 2002). Similarly, emergence of a new subgenotype C1 in Sarawak has resulted in the outbreak in 2003 (Podin et al, 2006). New variants of the virus can be generated from intra-typic and inter-typic recombination of the pre-existing viruses (Ding et al, 2009; Huang S.C. et al, 2008; Chan & AbuBakar, 2006). On the other hand, it must be stressed that emergence of a new genotype does not necessarily result in a large epidemic and adverse outcome. In a study comparing the fatal and non-fatal cases in Taiwan, it has been shown that the EV71 strains had a very high degree of genomic identity and only had a minor difference in the homology of the 3C protease (Shih et al, 2000). In a review done by Bible, it was concluded that linkage of virulence of the disease to genetic composition of the virus alone was not conclusive and the clinical outcome probably more related to the host-virus interaction and the immune response (Bible et al, 2007).

Table 3.1 Major outbreaks of HFMD in the world

Year	Country / Area	No. of persons affected	No. of deaths	Major complication(s)	Causative agent(s)	Ref
1969	USA (California)	20	1	Encephalitis, meningitis	EV71	Schmidt, Lennette & Ho, 1974
1972	USA (New York)	11	None	Aseptic meningitis, encephalitis	EV71	Deibel et al, 1975
1973	Sweden	195	None	Aseptic meningitis	EV71	Blomberg et al, 1974
1973	Japan	3296	N/A	N/A	EV71	Tagaya & Tachibana, 1975
1975	Bulgaria	705	68	Aseptic meningitis, bulbar encephalitis, paralytic disease	EV71	Shindarov et al, 1979
1977	USA (New York)	12	N/A	Aseptic meningitis, paralysis	EV71	Chonmaitree et al, 1981
1978	Hungary	1550	45	Aseptic meningitis, bulbar encephalitis, poliomyelitis	EV71	Nagy et al, 1982 Fujimoto et al, 2008
1978	Japan	36301	N/A	Brain stem meningitis, paralysis	EV71	Hagiwara, Tagaya & Yoneyama, 1978 Hagiawara et al, 1984

Year	Country / Area	No. of persons affected	No. of deaths	Major complication(s)	Causative agent(s)	Ref
1986	Australia	114	None	Aseptic meningitis, encephalitis, paralysis, respiratory diseases	EV71	Gilbert et al, 1988
1987	USA	45	None	Aseptic meningitis, encephalitis, paralysis	EV71	Alexander et al, 1994
1997	Japan	12	None	Meningoencephalitis, encephalitis, meningitis	EV71	Komatsu et al, 1999
1997	Malaysia	5999	≧35	Encephalomyelitis, cardiopulmonary failure	EV71 (CoxA16, A2, A4, A6, A9; CoxB5; EV1,4,5,7)	Chen T.C. et al, 2007 Herrero et al, 2003 Chua K.B. et al, 2007 Abubakar et al, 1999b WHO, 1997
1998	Taiwan	129106	78	>400 cases of aseptic meningitis, encephalitis, meningoencephalitis, acute flaccid paralysis, acute pulmonary oedema/haemorrhage	EV71 (CoxA16; CoxB1, B2, B3, B5; EV6,7,11,22,27)	Ho M. et al, 2000 Liu C.C. et al, 2000 Chen T.C. et al, 2007
1999	Australia	6000	N/A	Aseptic meningitis, Guillain-Barre syndrome, acute transverse myelitis, acute cerebellar ataxia, opso-myoclonus syndrome, benign intracranial hypertension, febrile convulsion	EV71	Sanders et al, 2006 McMinn et al, 2001
2000	Australia	200	N/A	Acute pulmonary oedema	EV71	Sanders et al, 2006

Year	Country / Area	No. of persons affected	No. of deaths	Major complication(s)	Causative agent(s)	Ref
2000	Singapore	3790	4	acute pulmonary oedema and haemorrhage, aseptic meningitis, encephalitis	EV71 (CoxA16, A3, A4, A5, A6, A10, A23, HEV18)	Chan K.P. et al, 2003
2000	Taiwan	80677	41	291 cases	EV71 (CoxA16,A9,A24; CoxB1, B3, B4; EV4, EV9)	Wang J.R. et al, 2002 Chen K.T. et al, 2007
2000	Korea	N/A	None	Aseptic meningitis, acute flaccid paralysis	EV71	Jee et al, 2003
2000	Japan (Hyogo)	60	1	Aseptic meningitis, cerebellar ataxia, acute flaccid paralysis, brainstem encephalitis	EV71	Fujimoto et al, 2002
2001	Taiwan	N/A	58	398 cases	EV71 (CoxA16,A6,A9,A24; CoxB4,B5; EV4, EV6)	Chen T.C. et al, 2007
2000	Malaysia (Sarawak)	169	N/A	Aseptic meningitis, acute flaccid paralysis, brainstem encephalitis	EV71 (CoxA16)	Podin et al, 2006
2000	Malaysia (Peninsular)	N/A	N/A	N/A	EV71	Herrero et al, 2003 Chua K.B. et al, 2007 Ooi M.H. et al, 2007b

Year	Country / Area	No. of persons affected	No. of deaths	Major complication(s)	Causative agent(s)	Ref
2003	Malaysia (Sarawak)	107	N/A	Aseptic meningitis, acute flaccid paralysis, brainstem encephalitis	EV71 (CoxA16)	Herrero et al, 2003 Chua K.B. et al, 2007
2005	Malaysia (Peninsular)	N/A	N/A	N/A	EV71	Chua K.B. et al, 2007
2005	Vietnam	173	N/A	Aseptic meningitis, acute flaccid paralysis, brainstem encephalitis	EV71	Tu et al, 2007
2006	Singapore	15282	N/A	1.8% of cases required hospitalization	EV71 (CoxA16)	Ang et al, 2009
2006	Malaysia (Sarawak)	291	6	Aseptic meningitis, brainstem encephalitis	EV71	Ooi M.H. et al, 2009
2006	Brunei	1681	3	Neurological complications	EV71	AbuBakar et al, 2009b
2007	China (Shandong)	1149	3	Aseptic meningitis, brainstem encephalitis	EV71	Zhang Y. et al, 2009
2008	China (Anhui)	488955	128	>353 severe cases. Neurogenic pulmonary oedema	EV71	Yang F. et al, 2008 WHO, 2009b
2008	Singapore	29686	1	4 severe cases of encephalitis	CoxA6, A10, EV71 (CoxA4,A16)	Wu Y. et al, 2010 Singapore Ministry of Health, 2008a

Year	Country / Area	No. of persons affected	No. of deaths	Major complication(s)	Causative agent(s)	Ref
2008	Taiwan	>255	N/A	Aseptic meningitis, encephalomyelitis, respiratory tract infection	CoxA16, B4, EV71 (CoxA2, A6, echovirus 4,30)	Hsu C.H. et al, 2011 Lee M.H. et al, 2011
2008	Hong Kong	967	1	8 cases with severe complications: aseptic meningitis, encephalitis, acute flaccid paralysis, pneumonia	CoxA16, EV71,B3 (CoxB1,2,4,5, echoviurs)	Tsui & Ma, 2008 Lau A. et al, 2008

1.4 Host susceptibility and molecular determinants of neruovirulence

HFMD is a common condition, especially in children under the age of 5 years. Seroprevalence study in Singapore showed that half of children aged 5 or older had antibody to EV71 which is a commonly identified virus causing HFMD in Singapore (Ooi et al, 2002). Infants less than 6 months are seldom affected. During the HFMD outbreak in Taiwan in 1998, the attack rate was lowest in seropositive infants aged less than 6 months, suggesting a protective role of maternal antibodies (Chang, et al, 2002; Ho M. et al, 1999). The presence of maternal antibodies of EV71 has also been demonstrated in neonates, the prevalence and titre of which correlate with levels in the mothers (Luo et al, 2009). Hence, it appears that the seroprevalence of neutralizing antibodies in women in childbearing age is important in protecting infants from HFMD. On the other hand, adults can also be infected and present with HFMD or even result in severe complications. In a retrospective analysis of cases admitted to public hospitals in Hong Kong from 2004 to 2009, there was an average annual number of about 50 cases of neurological conditions with positive laboratory results for enterovirus. Approximately 55% of them occurred in patients aged ≥15 years (Tam, 2010).

Extensive studies have been conducted in order to determine the virulence of EV71 based on the results of molecular genetics. 5'UTR and VP1 gene have been identified to account for difference in neurovirulence phenotype of polioviruses (De Jesus, 2007). Nevertheless, the genetic determinants of EV71 neurovirulence have remained elusive. In two outbreaks of HFMD occurring in Sarawak, either B4 or B5 were predominant, and a study showed children infected with B5 were more likely to have

neurological complications than those infected with B4 (Ooi et al, 2007b). Similarly, C1 genogroup viruses have been isolated from mild case of HFMD without complications while C2 genogroup viruses were accounted for severe infections of brainstem encephalitis and acute flaccid paralysis in Australia and Taiwan (McMinn, 2001a). The nucleotide sequence identity of the VP1 gene of genorgroups C1 and C2 is about 92%. Therefore, comparison of their complete genomic sequences may shed light on the location of neurovirulence determinants. Nonetheless, the complete genomic sequence of C1 genogroup nor such location has been identified so far.

It has been suggested that the virus-host interaction may be more important in determining the clinical severity of illness. Human leukocyte antigen-A33, which is more common in Asian populations than the white populations, has been postulated as one of the risk factor for EV71 infection (Chang et al, 2008). This might explain why HFMD is more common in the Asian populations than the Western countries. Partial cross-protection from previous infection is another factor affecting the pathogenesis. This explains why young age is associated with severe infections (Chang et al, 2002; Lu et al, 2002; Chang et al, 2004a)

1.5 Routes of transmission and transmission dynamics

HFMD is mainly transmitted by the faecal-oral route and respiratory droplets. Faeces and oropharyngeal secretions are important in the transmission of enteroviruses but the relative importance in their role for viral transmission is not exactly known. The period of communicability lies during the acute stage of the illness, but can be longer since shedding of enteroviruses in the stool can persist for 3 to 11 weeks after onset of

disease (Chung et al, 2001; Tosato, Rocchi & Archetti, 1975). Enteroviruses are also detectable in throat swabs but the duration of shedding from oral secretion is shorter (Chung P.W. et al, 2001; Tosato, Rocchi & Archetti, 1975). It has been demonstrated from laboratory that oropharyngeal secretions contained a higher viral load but a shorter duration of shedding than faeces (Wang J.R. et al, 2000; Ooi, 2007a). Furthermore, it is not uncommon for patients with enterovirus infection to present with respiratory tract symptoms. Chang et al had reviewed that 24% to 40% of patients infected with EV71 and Cox A16 also had respiratory symptoms of cough or rhinorrhea while only 6% to 10% had diarrhea (Chang et al, 1999a). In a recent review of HFMD outbreak in Taiwan in 2008, Chen et al also found similar results for EV71 and CoxA2 (Chen S.P. et al, 2010). Besides faecal-oral route and respiratory droplets, direct contact with open and weeping skin vesicles or contaminated objects may also transmit the viruses. The viruses may also be excreted in stools of infected patients for several weeks and can survive for days on fomites at room temperature.

The role of environmental factors in causing enterovirus infection is controversial. Outbreaks of picornavirus including Cox5, echovirus 9, 13 and 30 have been implicated in causing outbreaks with the sources traced to swimming pool or pond, and tap and bottled drinking water (Faustini et al, 2006; Centers for Disease Control and Prevention, United States, 2004; Hauri et al, 2005; Amvrosieva et al, 2006). In 2003 in Belarus, echovirus 30 and echovirus 6 have resulted in a large outbreak affecting 1222 children and over half of the patients developed meningitis (Amvrosieva et al, 2006). In the outbreak of Taiwan in 2998, usage of tap water was found to be a risk factor for HFMD or herpangina in univariate analysis (Chang et al, 2002). Viable enteroviruses like CoxB and echoviruses have also been isolated from

bottled and tap water (Lee S.H. & Kim, 2002; Vivier, Ehlers & Grabow, 2004; Ehlers, Grabow & Pavlov, 2005). Besides, clams, mussels, oysters and crabs have been identified to have enteroviruses, ranging from a prevalence of 8% to 40% (Ehlers, Grabow & Pavlov, 2005; Croci et al, 2000; Beuret, Baumgartner & Schluep, 2003; Dubois et al, 2004; Gabrieli et al, 2007). Nevertheless, foodborne outbreak of EV71 in humans has not been documented.

The reservoir of enteroviruses is only humans. Intra-familial transmission of HFMD occurs commonly. In a prospective cohort study in Taiwan, transmission rates from infected children to siblings were as high as 84% (Chang et al, 2004a). In the Taiwan epidemic in 1998, in addition to the intra-familial contact with HFMD cases, attendance at a kindergarten or a child care centre were significant risk factors associated with EV71 infection (Chang et al, 2002). This suggests that infected children are sources for further spread of the disease within the enclosed environment of schools through close contact.

1.6 Knowledge gap identified from literature

Given the fact that vaccine and chemoprophylaxis are still under development, prevention and control HFMD using non-pharmacological measures are the key areas of interest at this moment. Such public health strategies rest heavily on the thorough understanding the epidemiology of the disease. For example, the knowledge of incubation period of enetorvirus infection will help determining the surveillance period in an outbreak of HFMD in a child care centre. The following have been

identified as knowledge gap in studying the epidemiology, prevention and control of HFMD. First, the epidemiology of HFMD has been studied in many of the endemic areas including Taiwan, Singapore and Malaysia, but not well documented in Hong Kong. It is important to recognize the seasonal trend and cyclical pattern of HFMD, susceptible population and characteristics of outbreaks in Hong Kong. In particular, it is vital to assess whether there was any change in the epidemiological, clinical and laboratory characteristics of EV71 infections in 2008 epidemic since the Region-wide epidemic has caused much panic from both public and even health care professionals.

Secondly, associations between climate factors and various infections, particularly influenza, have been studied (Lowen et al, 2007; Zuk, Rakowski & Radomski, 2009a; du Prel et al, 2009; Chan PK et al, 2009; Shaman et al, 2009; Zuk, Rakowski & Radomski, 2009a). Chan has demonstrated that cold and humid conditions are associated with a higher activity of both influenza A and B in Hong Kong (Chan PK et al, 2009). However, little is known on this aspect for HFMD. The difference in global geographical distribution and seasonal difference within an endemic country suggest meteorological parameters play an important role in affecting HFMD activity. The concern on global warming further echoed the importance of studying whether such association exist. We need to determine which climate factors are associated with HFMD and their relative importance. Although we cannot change the weather in the environment, we can at least issue an early warning to the public if there is any lag time happening between the climate condition recorded and the diseases occurrence.

Thirdly, the transmission dynamics of HFMD in institutional outbreaks is not well

understood. There was no report studying the reproduction number for enteroviruses identified from literature. Such important parameter for emerging infectious diseases such as SARS and avian influenza have been estimated and helped better understand the disease transmission. The R_0 has played a central role in infectious disease epidemiology as it measures the infectiousness of a pathogen in a given population. Estimation of R_0 has been conducted for many emerging and re-emerging infectious diseases including SARS, avian influenza H5N1, Ebola virus, measles and Esherichia coli O157:H7 (Ward et al, 2009; Legrand et al, 2007; Laegreid & Keen, 2004; Mossong & Muller, 2000; Choi & Pak, 2003). R_0 is generally defined as the average number of secondary cases infected by a typical infected person in a population that is almost fully susceptible (Heffernan, Smith & Wahl, 2005). If an infection has a value of R_0 less than 1, the number of people infected by the disease decreases in each generation of infection and the possibility of developing into a major epidemic is small. If R_0 is greater than one, the number of people infected by the disease increases in each generation of infection, and the infection may be able to spread in a population. A larger value of R_0 generally implies an increased likelihood of an infection to spread through the population causing an epidemic. The determinants of R_0 include the number of contacts per unit time, probability of transmission per contact and the duration of infectious period (Ward et al, 2009). However, little is known from literature on the R_0 of enteroviruses causing HFMD.

Fourthly, public health measures such as personal hygiene campaign are often implemented but never evaluated for HFMD. Since there is currently no effective vaccine or chemoprophylaxis for controlling HFMD, public health actions rest on non-pharmacological measures. These include tighten awareness of the disease,

promoting personal hygiene such as covering nose and mouth when cough or sneezing, hand washing, cleansing and disinfection of toys and environment, social distancing and school closure. Such public health measures are often implemented before or during the seasonal epidemic but seldom evaluated. Little studies were performed to evaluate these control measures despite tremendous manpower and resource has been invested each year in various countries. One reason was the difficulty to identify suitable control group since more than often the epidemic would sweep over the whole territory or even the whole region. There is no well documented evidence on the effectiveness of school closure which has far-reaching social implications.

PART II :

STUDIES ON EPIDEMIOLOGY OF HFMD IN HONG KONG

Chapter 2. Study objectives and main data source for analysis

2.1 Aim and objectives

Aim

I analyzed the surveillance data of HFMD in Hong Kong with a view to better understand the disease epidemiology so as to guide public health actions. The present series of studies examined the epidemiological characteristics of HFMD in Hong Kong.

Objectives

The specific objectives of individual studies are:

Study (1) — characterizing the changing epidemiological features identified from various surveillance systems for HFMD

Study (2) — analyzing the characteristics of EV71 epidemic in 2008

Study (3) — examining the association between meteorological parameters and HFMD activity

Study (4) — determining the basic reproduction number of CoxA16 and EV71 using mathematical model

Study (5) — assessing the impact of SARS and influenza H1N1 on transmission of HFMD

Main data sources for research

2.2 Sentinel surveillance system for monitoring HFMD activity

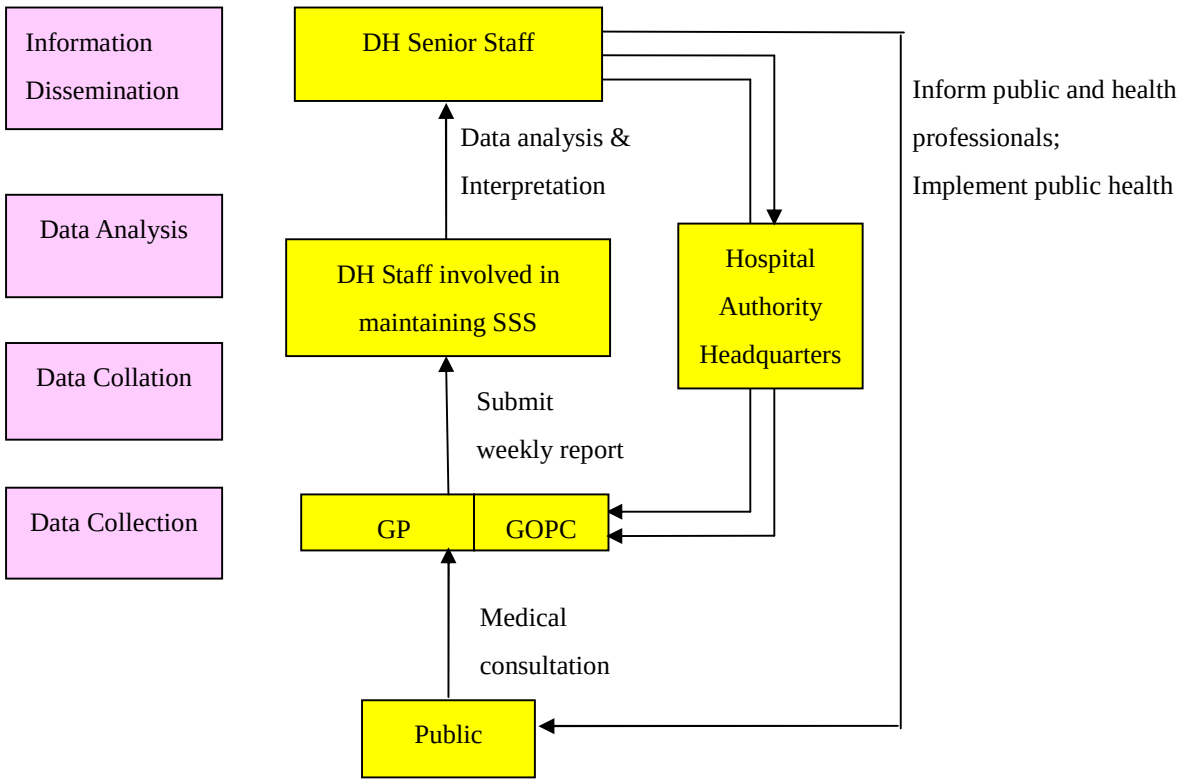
In view of the emerging condition, HFMD was added to the Sentinel Surveillance System (SSS) in Hong Kong since 1998. This system included sentinel clinics (64 GOPCs and about 40 GPs) distributed in all districts in Hong Kong. These GPs were in solo practice. Its main objectives were to:

- monitor the long-term trend of HFMD in Hong Kong;
- detect community epidemics early to provide warning signal and timely preventive measures; and
- contribute to a better understanding of its local epidemiology

2.2.1 Operation of the SSS for HFMD

The operation of the HFMD SSS is summarized in Figure 2.1.

Figure 2.1 Operation of the HFMD SSS in Hong Kong



2.2.2 Data collection

Every week, the sentinel doctors recorded the number of patients diagnosed with HFMD, according to a standard case definition:

1. Fever and
2. Small discrete papulovesicular lesions in oral cavity (enanthem); and
3. Sparse, grayish vesicles surrounded by erythematous base on hands or feet (exanthem).

Hence, there is no subclinical case. Laboratory confirmation of the pathogen causing the disease was not required. They recorded the age group breakdown of patients with HFMD and the total number of patients seen every week. Every Monday or Tuesday, they were required to complete the surveillance record form and return the forms to the DH by facsimile or email.

2.2.3 Data collation

The data were entered into an electronic database. The consultation rate of HFMD was calculated by dividing the number of patients diagnosed with HFMD by the total number of patients seen. The rate was expressed as the number of cases of HFMD per 1,000 consultations among GP and GOPC respectively.

2.2.4 Data analysis and interpretation

The public health physicians responsible for maintaining the SSS compared the figures with past consultation rates. The results were interpreted in conjunction with other surveillance systems such as hospital admission data.

2.2.5 Information dissemination

Every Friday, an internal report was sent to the senior staff of the DH while the

statistics were uploaded to website of the DH for public access. A weekly report was disseminated to all participating doctors via facsimile or email.

2.3 Institutional outbreaks of HFMD reported to DH

In Hong Kong, institutions such as child care centres and kindergartens are required to report suspected outbreaks of HFMD to DH for epidemiological investigation and control. Upon notification of the outbreak, prompt investigation will be conducted including case interview, site visit and laboratory investigation for causative agents accounting for the outbreaks. The management staff of the institutions will be advised on infection control measures. For each HFMD outbreak, the epidemiological investigation findings, as well as those clinical and laboratory results were entered into the electronic Public Health Information System.

2.4 EV 71 infection reported to Department of Health

Cases of EV71 infection were reported to DH voluntarily before it became a statutory notifiable since 6 March 2009. Detailed records of the EV71 cases reported to DH since 1998 were reviewed including the demographic characteristics, clinical presentation and outcome as well as the laboratory findings.

2.5 Laboratory surveillance for monitoring enteroviruses

Public Health Laboratory Centre of DH was the mainly reference laboratory for detection of enteroviruses in Hong Kong. Clinical specimens were tested for enterovirus upon referral from out-patient and in-patient settings in both public and private sectors. Enteroviruses were identified by culture of virus according to conventional method or detected of genome by in-house developed real-time reverse-transcriptase polymerase chain reaction.

Chapter 3. Study (1) -- Changing Epidemiology of Hand, Foot, and Mouth Disease in Hong Kong, 2001 to 2009

INTRODUCTION

There is currently no effective chemoprophylaxis or vaccination for HFMD or EV71 infection, public health interventions rest almost solely on non-pharmacological measures. Preventive strategies rest on a heighted public awareness about the disease, maintaining good body immunity to avoid being infected, and promotion of good personal hygiene practice such as covering nose and mouth when cough or sneezing and hand washing. Such measures should be maintained before and during the peak season of HFMD. When large community outbreak occurs, more stringent public health actions are required to halt disease transmission. These measures include stepping up personal hygiene, cleansing and disinfection, as well as social distancing such as isolation of the infected case from schools or even class suspension. A sensitive surveillance system is therefore imperative for detection of impending community outbreak early in order to issue early warning to the target population and guide public health actions.

The purposes for surveillance include tracking secular and seasonal trends of HFMD, control of individual outbreak especially in child care institutions, monitoring of severe infections for any change in disease morbidity or even mortality pattern, determination of predominant circulating strains of various enteroviruses or emergence of new genotypes of EV71. Different health authorities have different requirement and practice for surveillance for HFMD. In epidemic areas such as Taiwan, Mainland China, Singapore and Malaysia, either HFMD or other related

health conditions are notifiable disease by law. For example, in Mainland China, Singapore and Malaysia, HFMD is a statutory notifiable disease. On the contrary, doctors in Taiwan are required by law to report enterovirus infection with complication. This is complemented by the sentinel surveillance system for HFMD and herpangina set up at accident and emergency unit of hospitals, in order to better monitor the disease trends (Wu T.N. et al, 1999). In Malaysia, sentinel surveillance system for monitoring HFMD has also been set up in 1997 after outbreak occurred (Podin et al, 2006, Lum et al, 1998; Cardosa et al, 1999; WHO, 1997). It should be noted that HFMD is not a statutory notifiable disease in many Western developed areas including the United States, United Kingdom and various other European countries. In Hong Kong, HFMD is not a statutory notifiable disease. The activity of HFMD is monitored by various surveillance systems including sentinel surveillance, voluntary reporting of HFMD outbreaks and EV71 infection (until March 2009 when EV71 infection became a notifiable disease). I analyzed the surveillance data of HFMD in Hong Kong with a view to better understand the epidemiology of the disease, focusing on the time and patient characteristics, so as to guide public health preventive actions.

METHODS

Data collected from two independent surveillance systems from 2001 to 2009 were analyzed, namely, the sentinel surveillance and the outbreak reporting system. I analyzed the consultation rates of HFMD obtained from the SSS, which is expressed as the number of cases of HFMD per 1,000 consultations. I also analyzed the age group breakdown of patients with HFMD from the sentinel system. Besides, I also analyzed the trend of the same study period using the number of HFMD outbreaks

reported by the institutions. These institutions such as child care centre or kindergarten reported HFMD outbreaks voluntarily to DH for public health control. Pearson's correlation test was used to analyze whether the trends detected by these two independent systems correlate with each other, using Statistical Package for the Social Sciences, version 14.0, Chicago.

For each HFMD outbreak, the epidemiological investigation findings, as well as those clinical and laboratory results were entered into the electronic Public Health Information System. Since completed data for the investigation of these outbreaks was only available from 2001 to 2008, analysis of the details of HFMD outbreaks was confined to this period. Regarding spatial analysis, the geographical distribution of the HFMD outbreaks was reviewed according to the 18 administrative districts of Hong Kong. The number of schools (including child care centres, kindergartens, primary and secondary schools) in each administrative district in 2008 were obtained from Education Bureau and Social Welfare Department. I estimated the incidence for HFMD outbreaks in each district by dividing the average number of HFMD outbreaks reported during 2001 to 2008 by the number of schools in the corresponding district recorded in 2008. Mantel-Haenszel Chi-square test was used to examine whether there was any significant difference in type of institutions reporting HFMD over time.
I determined the age and sex distribution of affected persons of HFMD outbreaks recorded and analyzed any significant change of these characteristics over time using Mantel-Haenszel Chi-square test. The clinical presentation, hospitalization rate, complication rate and case fatality of all outbreaks reported in each year of the study period were also examined. During investigation of reported HFMD outbreaks, clinical specimens, e.g. stool collected from recently affected patients were sent to the Public Health Laboratory of DH for diagnosis of the causative agents.

Public Health Laboratory of DH was the mainly reference laboratory for detection of enteroviruses in Hong Kong. Enteroviruses were identified by culture of virus according to conventional method or detected of genome by in-house developed real-time reverse-transcriptase polymerase chain reaction. I reported the laboratory surveillance findings from 2001 to 2009. To assess the morbidity caused by EV71, I examined whether there was any association between the yearly proportion of EV71 among all enteroviruses detected by Public Health Laboratory and the average yearly hospitalization rate of HFMD patients reported in the outbreaks from 2001 to 2008. Linear regression was used to test for statistical significance.

RESULTS

Secular trend and seasonality

Figure 3.1 shows the trends of HFMD activities as reflected by the sentinel surveillance data and the number of outbreaks reported (Figure 3.1). The peaks and troughs of the two trends matched well with each other (Pearson's correlation coefficient = 0.73). Seasonal peak occurred from May through July each year except in 2003 when SARS epidemic occurred and in 2009 when Human Swine Influenza H1N1 pandemic occurred. From 2001 to 2009, about half (48.9%) of all HFMD outbreaks occurred during the peak season from May through July (Figure 3.2). Apart from the traditional summer peak, it was noted that a smaller winter peak also occurred from October to December which was more obvious since 2006. About one-quarter (27.3%) of the HFMD outbreaks occurred in the winter peak during this study period.

Geospatial distribution of HFMD outbreaks

Figure 3.3 showed the geographical distribution of HFMD outbreaks in Hong Kong, 2001-2008. The top three districts where HFMD outbreaks occurred most common were Kowloon City, Yuen Long and Shatin where more schools were located, reflecting districts with higher disease burden. On the other hand, there were districts showing higher incidence rate of HFMD outbreaks. The overall incidence for HFMD outbreaks was one in 15 schools per year. Four districts namely North, Kwun Tong, Yau Tsim Mong and Sai Kung had relatively higher incidence rate of HFMD outbreaks, ranging from one in ten to one in 13 schools per year. Schools located in these districts might worth further attention for preventive measures.

Demographic characteristics of persons affected

Majority (75.0%) of the outbreaks occurred in pre-primary institutions such as child care centres and kindergartens. Since 2003, more outbreaks were reported by education institutions of older age group, i.e. primary, secondary and tertiary schools. Mantel-Haenszel Chi-square test showed an increasing trend of HFMD outbreaks reported from these institutions ($p<0.001$). Similarly, there was a significant rising trend of older children diagnosed with HFMD by the sentinel doctors. Those aged older than 5 years accounted for only 25.4% in 2001 but increased to 33.0% in 2009 (Figure 3.4) ($p=0.01$, Mantel-Haenszel Chi-square test). Children of this age group were attending primary, secondary and tertiary schools. Nonetheless, children under 5 years old were predominantly infected (66.4%) while adults aged over 20 years accounted for only 2.6%. More boys were affected than girls but the male-to-female ratio did not change significantly over time ($p=0.22$, Mantel-Haenszel Chi-square test).

Clinical presentation and laboratory diagnosis

Data from HFMD outbreaks suggested most of the patients presented with a mild course of typical symptoms including fever, vesicles or rash on hand and foot as well as oral lesions (Table 3.1). Only 1.3% of the patients required hospital care. This hospitalization rate ranged from 0.6% to 2.8% in each year. The only patient with complication in the past 10 years that occurred in 2008 was a 5-year old boy who had good past health. He presented with high fever, oral lesion and rash over hands and feet. He developed neurological symptoms including ataxia and required intensive care. Stool sample was tested positive for EV71 by polymerase Chain reaction test. He attended a kindergarten cum nursery where an outbreak of HFMD was reported affecting a total of 20 children and a 28 years old teacher. The outbreak was controlled after temporary class suspension to stop the transmission. There was no fatal case reported among all patients reported from these 1121 outbreaks.

Regarding the laboratory investigation of causative agents accounting for the outbreaks, virus detection was made in 312 out of 1121 (27.8%) outbreaks. Overall, Coxsackie A (mostly Coxsackie A16) accounted for nearly 80% of the outbreaks while EV71 was the second commonest pathogen identified. Other causative agents included CoxB and some untyped enteroviruses.

An apparent association was observed between yearly proportion of EV71 detected among all the positive enteroviruses specimens detected by Public Health Laboratory, and the average yearly hospitalization rate of HFMD patients reported in the outbreaks (Figure 3.5). However, linear regression test only showed marginally significant result, $p=0.09$. When EV71 became more prevalent as the circulating virus, more HFMD cases required admission to hospitals for management.

DISCUSSION

Interpretation of major findings and relationship with published literature

Both the SSS and reporting of outbreaks revealed a consistent seasonal trend which allows public health authority to issue early warning to relevant parties. Similar reviews have been done in Belgium, Taiwan and Malaysia using the sentinel surveillance data demonstrating the viruses were more activity in summer seasons (Chen K.T. et al, 2007; Druyts-Voets, 1997; Podin, 2006). These systems are useful when large epidemic occurred so epidemiological characteristics of the disease could be compared with the past. Winter peak, from October to December, became more obvious in Hong Kong since 2006. It was interesting to know that similar observation has been reported from the surveillance findings in other areas including, Taiwan, Singapore and Belgium (Chen K.T. et al, 2007; Singapore Ministry of Health, 2010; Druyts-Voets, 1997). The exact reason for a more obvious winter peak found in recent years was not known. One postulation was the effect of warmer winter recorded in Hong Kong in the past few years. Data obtained from the Hong Kong Observatory showed the average of the last 12 weeks for mean daily temperate in 2006, 2007 and 2008 were 22.3°C, 21.7°C and 22.3°C respectively, slightly higher than that recorded from 2001 to 2003 (ranged from 21.6°C to 21.7°C). In fact, evidence had showed HFMD was associated with various meteorological factors including temperature, vapour pressure and duration of sunshine (Urashima, Shindo & Okabe, 2003). Further studies related to climatic factors and activities of enteroviruses are warrant in view of the emerging concern of effect of global warming on infectious diseases (Please see Chapter 5 for further information).

Similar to surveillance findings from other studies, children under 5 years old accounted for most of the HFMD cases (Bendig & Fleming, 1996; Chen K.T. et al,

2007; Druyts-Voets, 1997; Ang et al, 2009). For example, children below 5 years old in the United States accounted for three fourths of the EV71 cases (Khetsuriani et al, 2006). However, it is worth noticing that more teenagers were affected in recent years in Hong Kong. Such shift in susceptible population called for public health preventive actions to cover this target group as well. In Hong Kong, preventions against HFMD and EV71 infection, e.g. organizing health talks to embrace workers with infection control knowledge and skills, had also been extended to include primary and secondary schools in recent years.

Among the patients of the HFMD outbreaks, the hospitalization and complication rate was very low, 1.3% and 0.0% respectively. This was because CoxA virus accounted for a vast majority (nearly 80%) in causing the outbreak, and it was relatively mild illness compared with EV71. In contrast, if I examined EV71 infection alone, the complication rate would be much higher. In the epidemic of EV71 reported in 2008, 98 cases of EV71 were reported to the DH. 11.2% of the 98 cases had complications including meningitis or encephalitis (6.1%), pneumonia (3.1%), acute flaccid paralysis (1.0%), or shock (1.0%) (Ma et al, 2010a). In 2008, there was one fatal case affecting an 11-month-old boy and the case fatality rate for EV71 was 1.0%.

Cyclical high activity of EV71 was detected from this study. It is of paramount importance to monitor the laboratory results since EV71 is known to be associated with severe complications and fatality (Chang, 1999b; Lee T.C. et al, 2009; Huang C.C., 1999; Huang M.C., 2006; Ishimaru et al, 1980). In Japan, peak activity of EV71 occurs around every 3 to 4 years while the cyclical period in Malaysia seems to be shorter as 2 to 3 years (Hosoya et al, 2006; Podin et al, 2006). It has been suggested that the quiescence between epidemics is probably due to the development

of population immunity that occurs during the high-infection-rate epidemic (Hosoya et al, 2006). During the years of the quiescence of the virus, the accumulation of a susceptible population, especially in younger cohorts, probably contribute to the next epidemic (Bendig & Fleming, 1996; Chen K.T. et al, 2007; Khetsuriani et al, 2006).

Another interesting finding was the absence of summer peak in 2003 and 2009 when the pandemic of SARS and Human Swine Influenza (H1N1) occurred respectively. During these periods, overwhelming advice on personal hygiene such as handwashing and wearing face mask, had been promulgated everywhere in the territory for the whole summer. These were coupled with the effect of social distancing due to school closure which probably worked by reducing contact between sick and healthy children and hence transmission of viruses. Since CoxA virus and EV71 can also be transmitted through droplets, such public health measures in dealing with SARS and pandemic influenza also had "cross-over" protection against HFMD. In fact, evidence had shown that community hygiene measures had successfully reduced various infections spread by respiratory route during SARS epidemic (Lo et al, 2005). Similar observation was detected in Malaysia when the outbreak of EV71 occurred in 2003. The number of EV71 cases dropped considerably by end of April in Malaysia when public health measures for SARS put into place also served to mitigate the transmission of enteroviruses (Podin et al, 2006). Another possible explanation was shifting of attention from HFMD to SARS and pandemic influenza during these periods resulting in reduced reporting of HFMD cases. Pease see Chapter 7 for studying the impact of SARS and influenza H1N1 on transmission of HFMD.

Strengths and weaknesses of current study

To our best of knowledge, this is the most comprehensive review of HFMD situation

in Hong Kong, involving about 1200 outbreaks in reported in the past decade affecting over 8000 patients. This study helps us better understand the epidemiological characteristics of this common condition in Hong Kong. Moreover, we have studied two independent systems, namely the SSS and the outbreak reports but they showed good correlation and similar trends in characterizing the epidemiological features of HFMD. This has increased the validity of findings detected in this study.

However, it was noted that outbreaks of HFMD were reported on a voluntary basis and hence there might be under-reporting of such outbreaks that really happened. For example, both health care professionals and managers in child care centres and schools might be more alert about infectious disease after SARS occurred in 2003. They might report more cases and do more laboratory investigations since then. Nevertheless, it is unlikely that such under-reporting is different in summer and winter seasons, nor it would be different towards different population (age or sex) subgroups. Hence, such under-reporting would not affect the epidemiological trends detected in the present study.

Figure 3.1 Consultation rate of HFMD by SSS based at GP and number of HFMD outbreaks reported to DH, 2001 to 2009

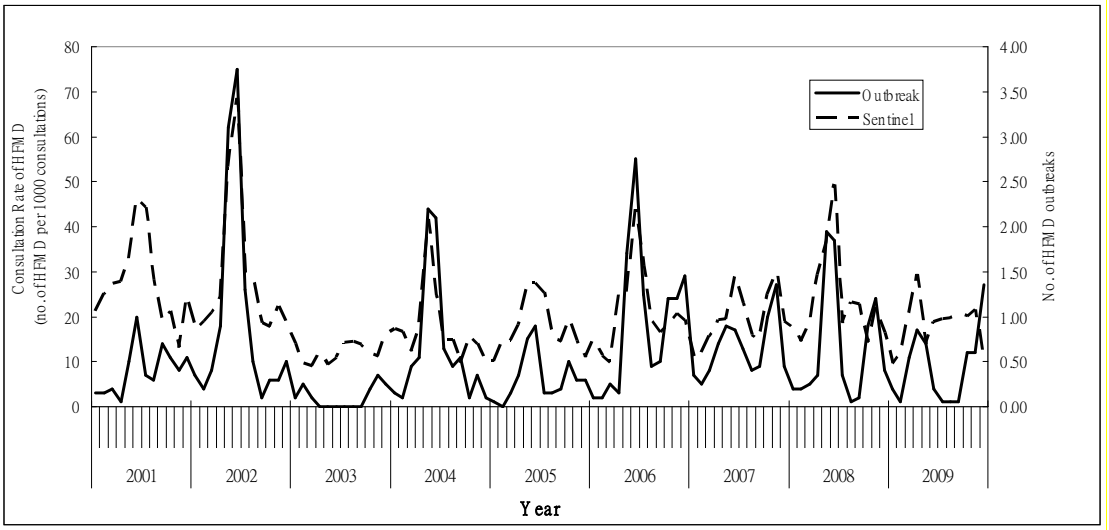


Figure 3.2 Number of HFMD outbreaks, reported to DH by month, 2001 to 2009

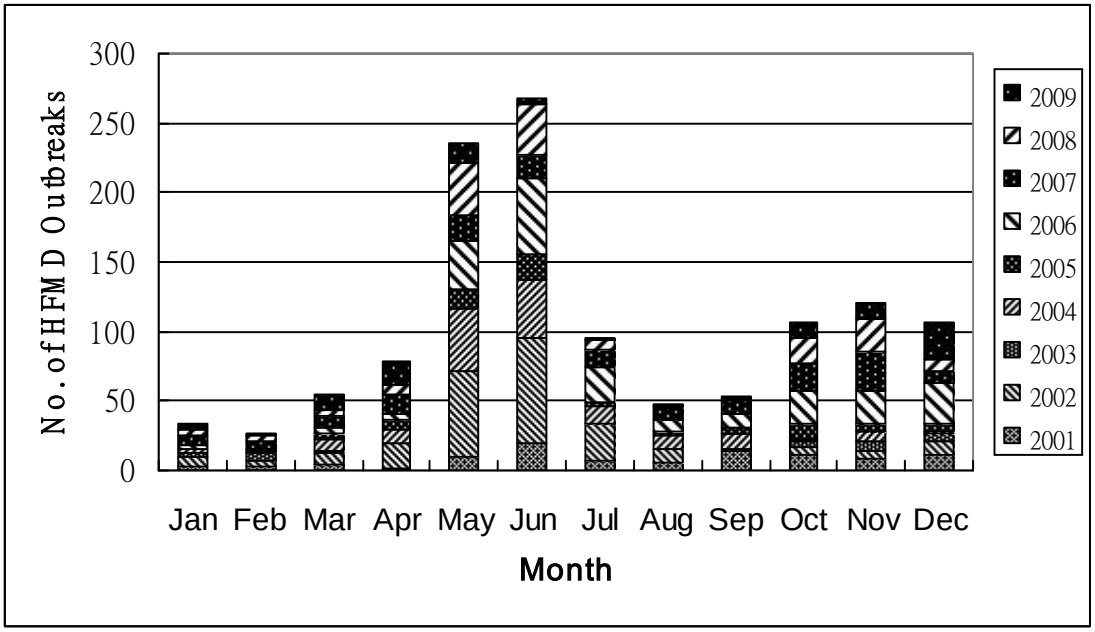


Figure 3.3 Geographical distribution of HFMD outbreaks in Hong Kong, 2001-2008

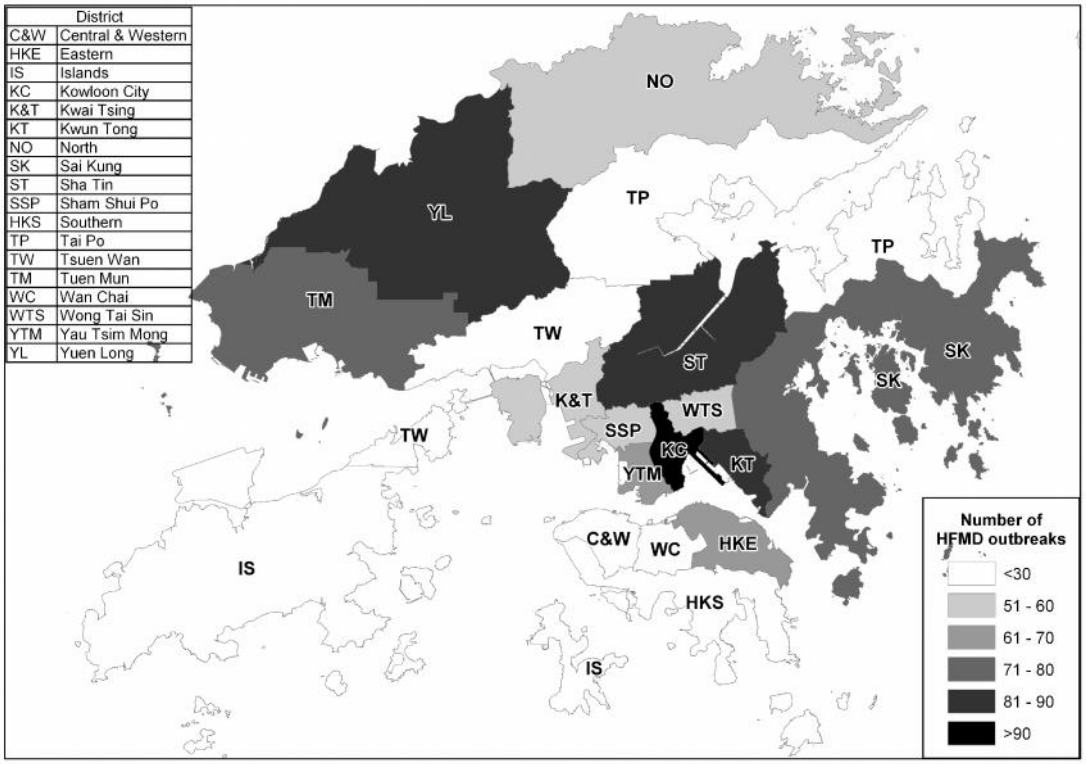


Figure 3.4 Age distribution of HFMD detected by SSS based at GP, 2001 to 2009 (N=3512 patients)

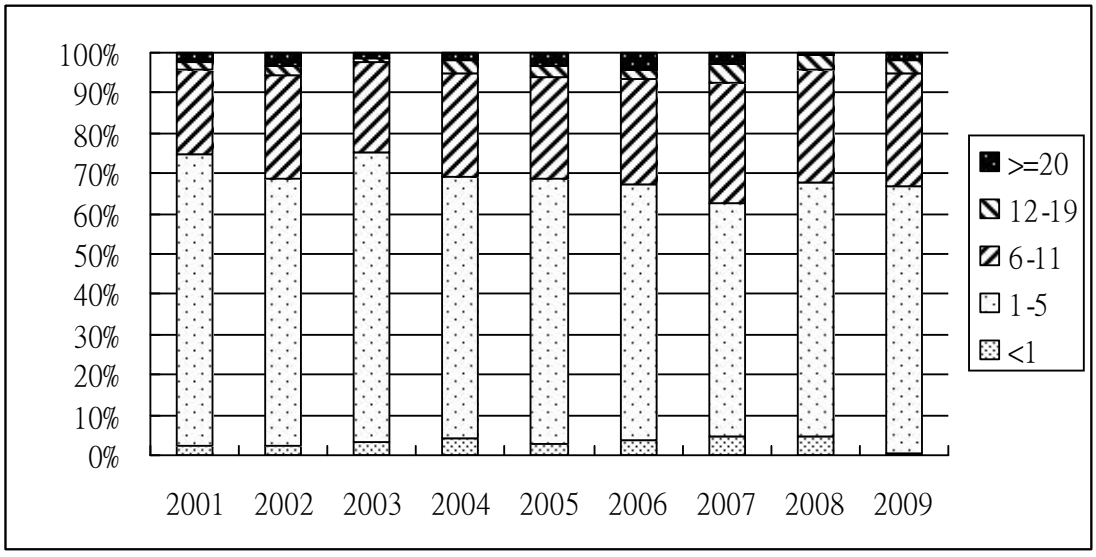


Figure 3.5 Linear regression plot of average yearly hospitalization rate of HFMD patients reported in the outbreaks against yearly proportion of EV71 detected among all enteroviruses, 2001-2008 (p=0.09)

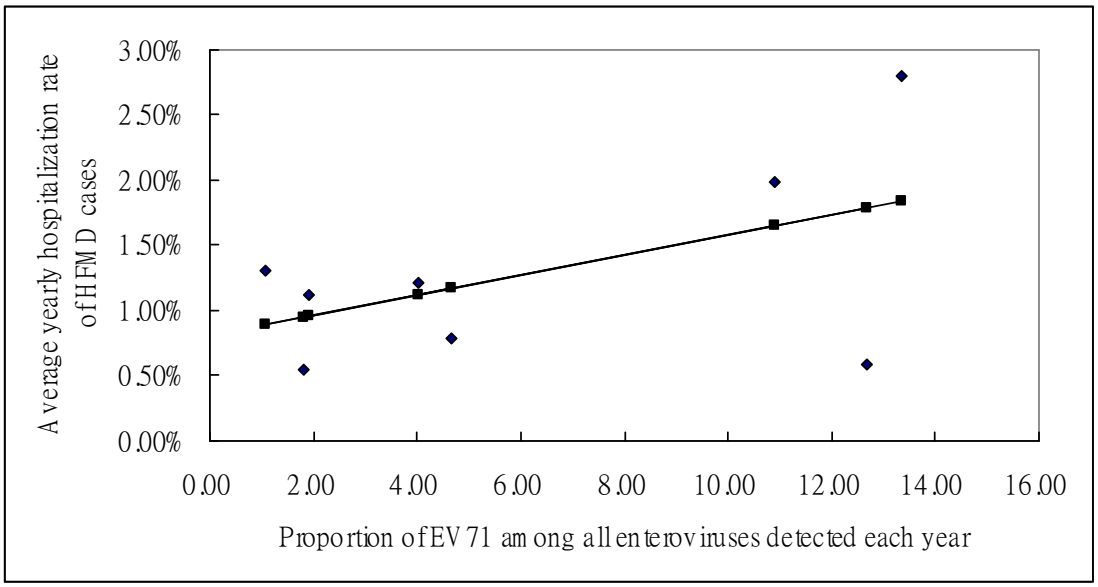


Table 3.1 Clinical and laboratory characteristics of HFMD outbreaks reported to DH, 2001 to 2008.

	2001	2002	2003	2004	2005	2006	2007	2008	Overall
No. of outbreaks	98	234	25	155	76	222	155	156	1121
No. of persons affected	804	1909	178	1034	545	1647	1072	893	8082
Clinical presentation (% of persons with symptoms and signs)									
Fever	43.8%	51.2%	3.4%	42.6%	30.5%	31.9%	41.4%	42.7%	40.7%
Vesicles on hand or foot	70.5%	54.4%	1.1%	49.2%	56.1%	61.1%	56.6%	51.3%	55.6%
Oral lesion	81.3%	88.9%	5.6%	76.2%	93.0%	91.0%	89.6%	88.4%	85.4%
Rash on face	0.0%	0.5%	0.0%	1.5%	0.9%	1.5%	0.7%	1.3%	0.9%
Rash on limb	31.1%	48.1%	3.9%	66.5%	65.7%	62.1%	61.9%	61.6%	55.2%
Rash on trunk	2.0%	1.8%	0.0%	5.0%	5.7%	4.0%	3.7%	5.3%	3.6%
Clinical outcome (% of persons with adverse outcome)									
Hospitalization rate	2.0%	1.3%	1.1%	0.6%	0.6%	0.8%	1.2%	2.8%	1.3%
Complication rate	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%
Fatality rate	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Laboratory testing (No. and % of outbreaks with laboratory results)									
Positive test	46	94	10	44	21	34	21	42	312
Coxsackie A virus	80.4%	94.7%	100.0%	65.9%	76.2%	88.2%	71.4%	50.0%	79.2%
Coxsackie B virus	0.0%	0.0%	0.0%	0.0%	0.0%	2.9%	0.0%	0.0%	0.3%
Enterovirus 71	15.2%	2.1%	0.0%	25.0%	14.3%	8.8%	28.6%	47.6%	16.7%
Other enterovirus	2.2%	0.0%	0.0%	4.5%	9.5%	0.0%	0.0%	0.0%	1.6%
Adenovirus	0.0%	0.0%	0.0%	2.3%	0.0%	0.0%	0.0%	0.0%	0.3%
Mixed	2.2%	3.2%	0.0%	2.3%	0.0%	0.0%	0.0%	2.4%	1.9%
Negative test	9	29	8	16	1	11	9	14	97
Test not done	43	111	7	95	54	177	125	100	712

Chapter 4. Study (2) -- Epidemic of enterovirus 71 in 2008 – its public health implication to Hong Kong

INTRODUCTION

EV 71 infection has became an emerging infection, especially in the Southeast Asia areas in the past few decades. Although most patients present with a mild illness of HFMD or herpangina, a small proportion of patients may suffer from severe complications of aseptic meningitis, encephalitis, acute flaccid paralysis, myocarditis or even death. (Ho M. et al, 1999; AbuBakar et al, 1999a; Shekhar et al 2005; Jan et al, 2000; Gilbert et al, 1988; Chong et al, 2003; Shah et al, 2003; Ho M., 2000). Young children are more susceptible and this is the reason why EV71 infection has captured much attention. A seroepidemiology study conducted in Singapore suggested that the age-specific seroprevalence rate reached a steady state at approximately 50% among children $\geq$5 years old (Ooi et al, 2002). Outbreaks occurring among these school-aged children have caused significant social disturbance to both children and their family. In Hong Kong and Singapore, some schools having outbreaks of HFMD associated with EV71 infection may require temporary class suspension or even school closure in order to stop the disease transmission.

Epidemics of EV71 have been reported worldwide since 1970's in Bulgaria, Hungary and Sweden with aseptic meningitis, brain stem encephalitis and significant numbers of death (Ho M, 2000; Blomberg et al, 1974; Shindarov et al, 1979). The epidemic wave has spread to Southeast Asia from 1997 to 2000 in Malaysia, Taiwan, Singapore and Japan (Ho M et al, 1999; AbuBakar et al, 1999; Chan KP, 2003; Fujimoto et al, 2002). The largest HFMD outbreak occurred in Taiwan in 1998 when over 100,000

cases of HFMD were reported, which were only less than 10% of the estimated true figure (Ho M et al, 1999). That outbreak resulted in 400 patients with severe illness and 78 deaths. EV71 was a particular concern since the virus had been isolated among 92% of those who died. In 2008, an epidemic of EV71 infection was detected again in many Southeast Asia areas including Hong Kong. I assessed the public health implication in Hong Kong by reviewing the epidemiological, clinical and laboratory characteristics of EV71 cases and compared them to past years' trends.

METHODS

In Hong Kong, EV71 was mainly diagnosed by the Public Health Laboratory of DH. Doctors are also requested to report EV71 infection to the DH on a voluntary basis. All cases diagnosed by Public Health Laboratory were being investigated. I identified cases from this reporting system from 1998 to 2008. A case was defined as a patient who had compatible clinical presentation and isolation of EV71 or detection of EV71 by polymerase chain reaction from a clinical specimen. Upon reporting of the EV71 cases, the patients or next of kin would be interviewed by trained public health doctors or nurses for epidemiological investigation and control, including contact tracing of home or other close contacts and controlling any associated HFMD outbreaks in the child care institutions. Clinical information including the onset date, clinical symptoms and signs, complication and outcome were obtained from the attending physician or hospital record. These data were retrieved through an electronic database which was reconstructed as the Public Health Information System since 2002.

I estimated the annual incidence rates in general population and different age groups. The incidence was calculated by the number of EV71 cases reported in 2008 divided by population data obtained from Census and Statistics Department (Census and Statistics Department, Hong Kong, 2009). The age specific incidence was estimated similarly using population of different age group. The monthly number of reported EV71 case was calculated to determine the seasonality.

The complication and case fatality rates were calculated by dividing the number of cases who had complications and the number of fatal cases respectively by the total number of cases reported. These rates calculated from cases reported in 2008 were compared with the corresponding rates calculated from cases reported from the period 1998 to 2007, in order to assess whether the virus was more virulent and associated with more severe clinical presentation and outcome. Besides, I also compared the % of cases requiring hospitalization, median duration of hospital care, and the likelihood of being associated with an HMFD outbreak in institution. These rates were compared using Chi-square test with Yate's correction whenever appropriate. The Mann-Whitney U test was used for statistical calculation to compare duration of hospital stay which was left-skewed.

The laboratory diagnosed was confirmed by either isolation of EV71 or detection of EV71 by polymerase chain reaction from a clinical specimen. Virus isolation was performed according to conventional method and EV71 strains were detected by in-house developed real-time reverse-transcriptase polymerase chain reaction. Phylogenetic tree was constructed by using the neighbour-joining method. The

molecular epidemiology of EV71 detected in 2008 was compared with the past years' trends.

RESULTS

Ninety-eight EV71 cases were reported in 2008, highest in the past decade (Figure 4.1). The estimated annual incidence was 1.4/100 000 in the general population, with the highest incidence reported in children aged 0-4 years old (27.9/100 000) (Figure 4.2). The seasonal peak was detected from May through July, contributing to 65.3% of the cases while a small winter peak (October to December) was also noted which was not seen in the past 10 years (Figure 4.3).

More males were affected with a male-to-female ratio of 1.1:1. The age of the patients ranged from 5 months to 42 years with a median age of 3.5 years (Table 4.1). Seventy-one cases (72.4%) were aged five years old or less. It was noticed that EV71 also affected four adults (aged from 24 to 42 years), accounting for 4.1%. Among these four adult patients, three were family members of the paediatric cases and they were identified during contact tracing. The remaining adult patient also had contact history of a child with HFMD although no laboratory diagnosis was made for that child.

Majority (90.8%) of the cases presented with HFMD while 2 cases suffered from milder course of herpangina. Others presented with non-specific symptoms including

fever, upper respiratory illness, non-specific skin rash, pneumonia. Sixty-one cases (62.2%) required hospital care with a median duration of stay for 4 days. Majority of the cases recovered uneventfully while 11.2% of the cases had complications including meningitis or encephalitis (6.1%), pneumonia (3.1%), acute flaccid paralysis (1.0%), and shock (1.0%). There was only one fatal case and the CFR was 1.0%. This affected an 11-month-old boy who had good past health and presented with fever and shortness of breath in August 2008. His clinical condition deteriorated quickly and was certified dead at the emergency department despite resuscitation. The cause of death was attributed to interstitial pneumonitis and the throat swab, tracheal aspirate and rectal swab were all positive for EV71 by polymerase chain reaction. His three-year old elder sister was also diagnosed to have EV71 infection but she recovered without any complication.

Among the 61 cases who were known to attend schools or child care institutions, 28 (45.9%) of them had concurrent HFMD outbreaks in their schools or institutions. These 21 HFMD outbreaks affected a range of 2 to 25 persons. Most outbreaks could be controlled with stepped up infection control measures including observing strict personal hygiene by frequent handwashing, thorough cleansing and disinfection of the contaminated surface and soiled items, and excluding children with HFMD from schools until fever has subsided and all the vesicular lesions have dried and crusted. However, there was evidence of sustained transmission of EV71 in six schools despite implementation of these stepped up measures. They required temporally class suspension of 14 days in order to halt the disease transmission.

Both the complication rate and case fatality rates were statistically not significant when compared with the corresponding rates of the past 10 years (p-value = 0.12 and 1.00 respectively). The EV71 cases reported in 2008 showed no statistical difference in either the % requiring hospital care or the duration of stay in hospital. Regarding the proportion of associated HFMD outbreak, the cases reported in 2008 was apparently less likely to be associated with an HFMD outbreak when compared with the relevant figures in the past decade. It was noted that a significantly higher proportion of cases in 2008 reported a travel history outside Hong Kong (mainly Mainland China) during the incubation period. However, it was difficult to ascertain the exact source of infection for these cases as EV71 infection is endemic in both Hong Kong and other areas where the cases had visited.

Figure 4.4 illustrated the result of phylogenetic analysis from 1998 to 2008. It was found that majority of the cases reported in 2008 were C4 strains, which was also the predominant circulating strains in the past ten years. C1, C2, B3 and B4 strains were detected in previous years but only constituted a small portion. In 2008, the rectal swab of a Taiwan tourist was positive for EV71 by polymerase chain reaction and gene sequencing results showed it was B5 strain. The fatal case reported in 2008 belonged to C4 while the other two fatal cases were C4 (reported in 2000) and B3 (reported in 1999) respectively.

DISCUSSION

Interpretation of major findings and relationship with published literature

Our estimated incidence of EV71 infection in Hong Kong was 1.4/100 000 in the general population, with the highest incidence reported in children aged 0-4 years old (27.9/100 000). The true incidence was probably much higher because many patients might present with only mild HFMD and managed at out-patient settings and were not tested. The bias due to under-reporting by doctors was considered to be minimal since not all laboratories in Hong Kong have the capacity to isolate the EV71 virus or detect EV71 by real-time polymerase chain reaction. In fact, the Public Health Laboratory of the DH is the reference laboratory, which receives referral specimens from public hospitals or private practitioners. Essentially, all EV71 cases were diagnosed by the Public Health Laboratory and the current review has captured all laboratory confirmed cases.

Apart from the traditional peak season of EV71 detected in summer months, I also noticed that there was a rise in EV71 activity in 2008 winter months from October to December. This was consistent with the findings of other surveillance systems for HFMD reported in Hong Kong since 2006, including the number of institutional HFMD outbreaks reported to the DH, the SSS based at GP and child care centres (Centre for Health Protection, Hong Kong, 2009). EV71 has contributed to a higher proportion of the circulating enteroviruses throughout the whole year of 2008 compared to 2006 and 2007, although CoxA viruses were still predominant according to the laboratory surveillance in these three years. It is interesting to note that such a bimodal peak for HFMD was also observed in other Asian countries including Singapore but not in Taiwan in recent years (Singapore Ministry of Health, 2008b; Taiwan Centers for Disease Control, 2009).

It was often worrying that a high circulation of EV71 would result in worse clinical presentation and outcome. In the epidemic of HFMD in Taiwan in 1998, EV71 was isolated in 78 patients with various complications and 34 of the fatal cases (Ho M, et al, 1999). Our analysis showed that both the complication rate and case fatality rates were not statistically significant compared with the past years' data. The complication rate in 2008 was 11.2%, higher than the average of 6.2% during the period of 1998 to 2007. A similar higher proportion of cases required hospitalization in 2008 (62.2% in 2008 vs 50.3% in 1998-2007). This might be partly attributed to the enhanced hospital-based surveillance system for HFMD implemented in early May 2008 in Hong Kong in view of the HFMD outbreak occurring in Mainland China (Chinese Center for Disease Control and Prevention & WHO, 2008). To facilitate early detection of severe infections of HFMD and implement public health actions, doctors in Hong Kong were requested to report clinical cases of HFMD with severe complications admitted to hospitals since May 2008, even without laboratory confirmation. Among the 98 cases reported in 2008, 5 cases were reported through this system and were subsequently confirmed to be EV71 infection. Subtracting these 5 cases, the complication rate in 2008 would be 6.5% (6/93), similar to the rate (6.2%) estimated during the period of 1998 to 2007.

In 2008, majority (63.6%) of the cases with clinical complications were children 5 years or younger. Meningitis and encephalitis were the most commonly reported complications. One patient suffered from acute flaccid paralysis which was also seen in the outbreak in Perth, Australia in 1999 and the Taiwan outbreak in 1998 (Ho M., et al, 1999; McMinn, Stratov & Dowse, 1999). In contrast to the previous two reported fatal cases in Hong Kong, the fatal case reported in 2008 did not have any

documented evidence of central nervous system complication. In the first fatal case reported in 1999, a 2-year-old boy suffered from brainstem encephalomyelitis with rapid cardiovascular decompensation (Ng et al, 2001). The second fatal case reported in 2000 resembled the death cases experienced in Taiwan in the 1998 outbreak. It affected a 5 year-old girl who presented with meningoencephalitis, and complicated by pulmonary oedema and pulmonary haemorrhage. The cause of death for the 2008 case was interstitial pneumonitis. Pulmonary complications due to EV71, although rare, are also reported in the literature (Shah et al, 2003). After reviewing the 78 cases with severe infections caused by EV71 in Taiwan, Ho found that there were 9 cases had pulmonary oedema or hemorrhage without any neurological complications (Ho M. et al, 1999).

Our epidemiological data suggested that there was a 3- to 4-year cycle of high activity of EV71 infection in Hong Kong. Similar surveillance findings were reported in Malaysia, Taiwan and Japan (Podin et al, 2006; Chen K.T. et al, 2007; Hosoya et al, 2006). In fact, it has been suggested that there were three separate waves of EV71 activities in the world, one in each decade between 1970 to 2000, with the last wave occurring from 1997 to 2000 (Bible et al, 2007). Interestingly, about ten years from the last wave, an epidemic of EV71 occurred again in 2008 when many Asian areas including Malaysia, Singapore, Taiwan and Mainland China reported high activity of the disease. There are different postulations why the epidemics occur cyclically. The emergence of new circulating strain was many a time a focus (Khetsuriani et al, 2006). However, the present findings did not suggest that there was a major change in molecular epidemiology of EV71 in Hong Kong. The predominant circulating strain remained as C4 in Hong Kong in 2008. Another postulation for the cyclical high

activity was the accumulation of susceptible population. Before the large epidemic in Taiwan in 1998, Lu had shown that from 1989 to 1993, 40% to some 60% of the study population was seropositive for EV71 (Lu et al, 2002). However, in 1994 and 1997, a large drop in seroprevalence (20% to some 30%) of EV71 infection was detected in the community. Such an accumulation of non-immune population may facilitate a more efficient transmission of the virus in the community.

It was noted in 2008, the proportion of cases with travel history outside Hong Kong during incubation period was 39.8% (39 cases) compared with the average of 10.3% in the period 1998-2007 (Table 4.1). Among the 39 cases reported in 2008, 38 cases traveled to Mainland China. There was a chance of increased infections in Hong Kong due to imported cases from China in 2008. However, since there were also plenty of local cases throughout the whole year in Hong Kong, one cannot commit to the source of infection of these cases, i.e. they could either acquire the infection from either Mainland or in Hong Kong. Having a travel history in Mainland did not necessarily mean that they were infected from others in China. In fact, very often, the exact source of infection cannot be traced during the epidemiological investigation.

Strengths and weaknesses of current study

The present study provides a comprehensive review of EV71 cases over a long period of time (1998 to 2008) in Hong Kong. This review includes analysis of epidemiological, clinical presentation, complication, mortality and molecular data of EV71 infections. Clinicians would have a better understanding of this emerging infection with local data. In particular, the results of phytogenetic tree (Figure 4.4) provide useful information on how this infection has evolved.

Since EV71 infection was reported only on a voluntary basis, there might be under-reporting of this disease. However, as mentioned earlier, Public Health Laboratory Centre would capture nearly all EV71 cases. The reporting bias would be minimal.

Figure 4.1 Number of enterovirus 71 cases reported to DH by year, 1998 to 2008

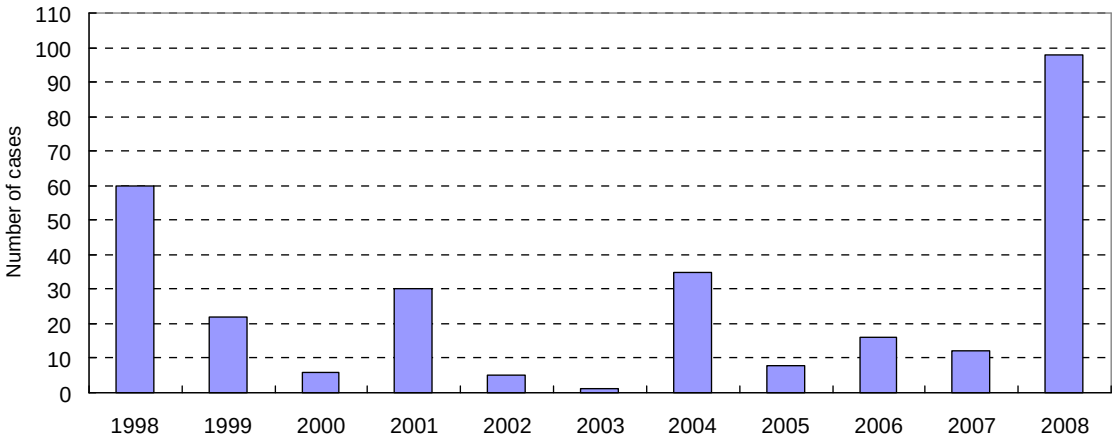


Figure 4.2 Age-specific incidence of enterovirus 71 cases in 2008

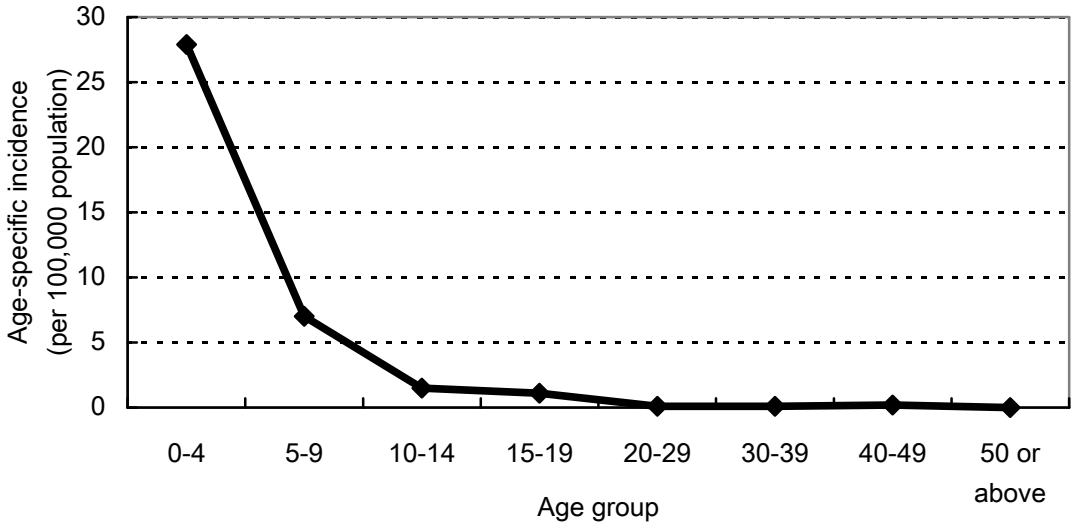


Figure 4.3 Number of EV71 cases reported to DH by month, 1998 to 2008

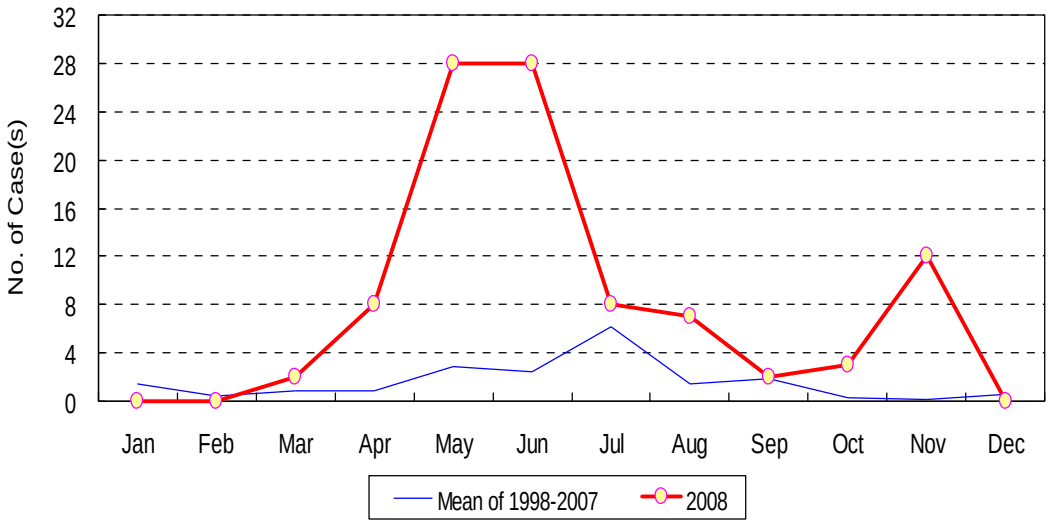


Figure 4.4 Phylogenetic analysis of EV71 cases in Hong Kong, 1998 to 2008

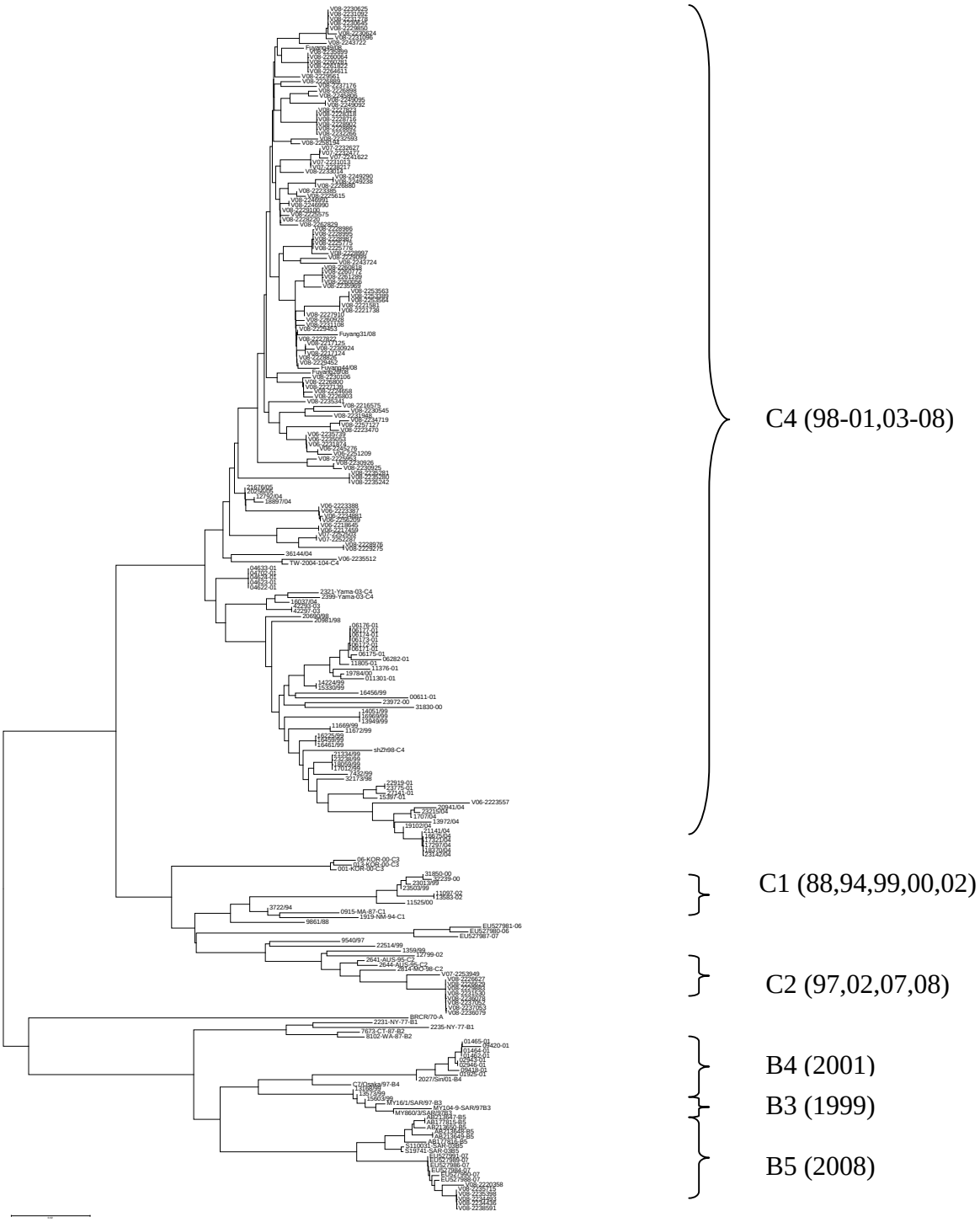


Table 4.1 Characteristics of EV71 cases in 2008, compared with cases reported, 1998 to 2007

	2008	**1998-2007**	P-value
No. of cases	98	195	-
Age distribution ≤ 12 years old (%) ≤ 5 years old (%)	 88 (89.8%) 71 (72.4%)	 183 (94.3%) 156 (80.4%)	 0.21 0.14
Male to female ratio	1.1:1 (52:46)	1.6 :1 (118:76)	0.20
No. of cases with travel history outside Hong Kong during incubation period (%)	39 (39.8%) Mainland (38) Taiwan (1)	20 (10.3%) Mainland (19) Macao (1)	0.00
Clinical presentation	HFMD: 89 Herpangina: 2 Others†: 7	HFMD: 185 Herpangina: 4 Others: 6	0.28
No. of cases required hospital care (%)	61 (62.2%)	98 (50.3%)	0.07
Duration of hospital care in days: range (median‡)	1 to 42 (4)	1 to 28 (4)	0.80
No. of cases with complication	11 (11.2%) Meningitis / encephalitis (6) Acute flaccid paralysis (1) Pneumonia (3) Shock (1)	12 (6.2%) Meningitis / encephalitis (10) Acute cerebellar ataxia (1) Pneumonia (1)	0.13
No. of death (Case fatality rate)	1 (1.0%)	2 (1.0%)	1.00
No. of cases associated with HFMD outbreak in school (% among school age cases)	28 (28.6%)	83 (42.6%)	0.02

†Include fever, upper respiratory infection, rash, pneumonia

‡Four missing cases in the group 1998- 2007 were excluded from analysis

Chapter 5. Study (3) -- Is Hand, Foot, Mouth Disease associated with meteorological Parameters ?

INTRODUCTION

The cyclical rise in activity of HFMD has caught much international concern and hence health authorities need to closely monitor the disease activity through various surveillance systems. Associations between climate factors and various infections, particularly influenza, have been studied (Lowen et al, 2007; Zuk, Rakowski and Radomski, 2009; du Prel et al, 2009; Chan PK, et al, 2009). Chan has demonstrated that cold and humid conditions were associated with a higher activity of both influenza A and B in Hong Kong (Chan PK, et al, 2009). However, little is known on this aspect for HFMD. The objective of the present study was to examine the relationship between climate change as measured by various meteorological parameters and HFMD activity as reflected by the consultation rate of HFMD diagnosed by general practitioners participating in the sentinel surveillance system in Hong Kong. Though this study, I hope to better understand the epidemiology of HFMD, which in turn assist in formulating the preventive strategies.

METHODS

Study design

This was a retrospective study examining the relationship between the meteorological parameters and HFMD disease activities in the community as indicated by the

consultation rates of HMFD calculated through the SSS. The data collected in the past decade, from 2000 to 2009 were divided into two study periods. For the first half of the study period, I examined the data collected from 2000 to 2004 to explore whether there was any association between the meteorological parameters and HFMD consultation rates, and developed the model explaining the trend of the consultation. For the second period from 2005 to 2009, I used the established model to predict the HFMD consultation rates, and compared the observed and the predicted rates.

HFMD activity

The sentinel surveillance system is a well established system developed by the DH to monitor various infectious diseases including HFMD. The consultation rate of HFMD was calculated by first summing up the total number of HFMD cases seen by all sentinel doctors and then divided by the total number of consultations of these doctors. This weekly consultation rate was expressed as the number of HFMD cases per 1000 consultations.

Meteorological parameters

The meteorological data collected during 2000 to 2009 were obtained from the Hong Kong Observatory. These included mean temperature, relative humidity, total rainfall, atmospheric pressure, wind speed, solar radiation and other parameters. Daily diurnal variation in temperature was calculated by subtracting minimum from maximum temperature. Table 5.1 summarizes all the meteorological parameters studied and the

value used for testing the association. For example, the weekly mean of maximum temperature was calculated by averaging the daily maximum temperature of a week while total rainfall was calculated by summing up the amount of rainfall measured for the whole week.

Data analysis

I examined for any association between HFMD consultation rates and each meteorological parameter using Spearman's rank correlation. Since different meteorological parameters might be correlated with each other, I further tested for any correlation among the meteorological parameters using Pearson's or Spearman's rank correlation whichever appropriate. Including two strongly collinear independent variables in a regression model may potentially lead to an erroneous conclusion that there is no association with the outcome variable even if they really have (Tormod & Bjørn-Helge, 2001). This occurs when collinearity is high enough to dramatically increase the standard errors of the coefficients. In order to adjust for any effect of correlation between meteorological parameters on HFMD activity, the meteorological parameters were entered into the multiple linear regression models. Forward stepwise approach was used in the regression model by adding meteorological parameter step by step until the best fit model was found. I reported the partial correlation coefficient and their 95% confidence interval (upper and lower limits) of each parameter in the model. Furthermore, theoretically if the activity of HFMD was indeed affected by the change in climate conditions, the change in HFMD consultation rates should lag behind the meteorological parameters, taking into account of the incubation period of HFMD and the delay in seeking medical attention. To adjust for these factors, I

repeated the regression analysis using HFMD data with 1 week, 2 weeks and 3 weeks lag time, given the understanding that incubation period for coxsackie viruses, common pathogens for causing HFMD, were about one week (Heymann D, 2004). During the epidemic of Severe Acute Respiratory Syndrome (SARS) in 2003, transmission of respiratory viruses in Hong Kong was greatly reduced by massive use of face masks and school closure (Lo et al, 2005). I also performed the regression analysis after excluding the data collected during the SARS period. Statistical Package for the Social Sciences, version 14.0, Chicago was used for analysis. Two-tailed analysis were used for all statistical tests and *P* values less than 0.05 were considered to be statistical significant.

In the second half of the study, the model constructed from the above analysis was used to predict the HFMD consultation rates from 2005 to 2009. I compared the actual and predicted values of HFMD consultation rates using Spearman's rank correlation. The prediction was repeated after excluding the data collected during pandemic influenza H1N1 in summer of 2009, due to similar reasons mentioned above for the SARS period. I also performed sensitivity analysis to examine how the HFMD consultation rates varied if I changed the estimates of the partial correlation coefficient of the meteorological parameters, using their upper and lower limits found in the regression model.

RESULTS

The trend of HFMD consultation rate from 2000 to 2009 is shown in Figure 5.1. It

was found that a seasonal peak was found for summer months of each year except in 2003 and 2009 when SARS and the pandemic influenza H1N1 occurred respectively. Interestingly, a smaller winter peak was also noted in the last 12 weeks (around October to December) of the years since 2006.

For the individual meteorological parameter, HFMD consultation rates were positively associated with maximum temperature, mean temperature, minimum temperature, diurnal difference in temperature, dew point, relative humidity, evaporation and total rainfall, and was negatively associated with atmospheric pressure (Table 5.2). Among these factors, maximum temperature, dew point and atmospheric pressure had the strongest association with Spearman's rank correlation co-efficient ranging from 0.261 to 0.334. Total sunshine and wind speed were not significantly associated with HFMD consultation rates.

Various meteorological parameters were found to be inter-related to each other. Table 5.3 summarized the correlation coefficients between all these parameters. It was found that mean temperature, maximum temperature, minimum temperature, dew point, atmospheric pressure and evaporation were highly correlated with each other, with correlation coefficient higher than 0.80. Besides, total bright sunshine and solar radiation were also highly inter-related with correlation coefficient being 0.88. Hence, I included only mean temperature and solar radiation in the regression analysis model, and excluded other highly correlated variables. Apart from these two variables, other variables included in the regression analysis were relative humidity, diurnal difference in temperature, total rainfall and wind speed.

Table 5.4 shows the results of linear regression analysis to explain the HFMD consultation rates during 2000-2004. In the M0 model, which had no lag time for analyzing the HFMD consultation rates and the climate parameters, HFMD consultation rates were shown to be positively associated with mean temperature and rainfall, after adjusting the effect of other parameters. When the lag time was taken into consideration, the M2 model was a better fitted one, with higher R^2 of 0.119 than the M0 and M1 models which R^2 were 0.079 and 0.062 respectively. This indicated that HFMD consultation rates were better explained using meteorological parameters measured 2 weeks earlier. In this M2 model, mean temperature, diurnal difference in temperature, relative humidity and wind speed were positively associated with HFMD consultation rate. The R^2 of M2 model would be even higher (0.154) if I excluded the period of SARS in 2003. The M3 model showed similar results with a slightly higher R^2 of 0.122.

Taking into account that the incubation period for enteroviruses causing HFMD was about one week and assuming the patients sought medical consultation a couple of days after clinical presentation, I estimated the lag time between climate parameters and HFMD consultation rates to be two weeks. In addition, the R^2 of M3 model was actually more or less the same as that of the M2 model. Hence, I used M2 model for our prediction of HFMD consultation rates for the year 2005 to 2009. There was statistically significant association between the predicted HFMD consultation rates and the observed one with Spearman's rank correlation coefficient being 0.276, P = 0.000 (Figure 5.2). However, it was noted in summer months of 2009 when pandemic influenza H1N1 occurred, the observed HFMD consultation rates were lower than that of the predicted ones. If the data during this period were excluded, I could obtain a

better correlation (Spearman's rank correlation coefficient of 0.298 vs 0.276) between the predicted and the observed HFMD consultation rates. The results of the sensitivity analysis are shown in Figure 5.3a-3d illustrating how the estimated HFMD consultation rates change if I varied the partial coefficients of mean temperature, diurnal difference in temperature, relative humidity and wind speed. It was noted that HFMD consultation rates were mostly affected by varying the relative humidity while it was least affected by wind speed.

DISCUSSION

Interpretation of major findings and relationship with published literature

Although non-polio enteroviruses are distributed worldwide, the prevalence varies with time and place. In countries with temperature climates, epidemics tend to occur in summer and autumn months while the infections are common throughout the year in tropical regions (Bendig & Fleming, 1996; Centers for Disease Control and Prevention, United States, 2000; Druyts-Voets, 1997; Chen K.T., 2007; Ang et al, 2009; Podin et al, 2006). Hong Kong is situated at latitude of 22.5° North with sub-tropical, tending towards temperate for nearly half the year. It is hot and humid in summer months and afternoon temperatures often exceed 31°C whereas at night, temperatures generally remain around 26°C with high humidity (Hong Kong Observatory, 2010). The seasonality of HFMD detected by the SSS in Hong Kong is similar to the patterns seen in other areas of the region (Chen K.T., 2007; Ang et al, 2009; Podin et al, 2006). The present study shows HFMD activities is significantly associated with different meteorological parameters. Increasing the temperature and relative humidity would increase the activities of HFMD while increasing the

atmospheric pressure would decrease its activities. The mean temperature, diurnal difference in temperature, relative humidity and wind speed are the most important factors after adjusting the correlation between the meteorological parameters. The HFMD trends are better explained if I use meteorological parameters measured two weeks before the clinical consultation rate of HFMD reported to the health authority. This is compatible with the understanding of the incubation period and possible delay in seeking medical care by the patients. In Hong Kong, most of the viruses causing HFMD are Coxsackie A16, and occasionally other CoxA subtypes, CoxB viruses or EV71. The incubation period for these enteroviruses are about three to five days (Heymann D, 2004). This explains why the M2 model has a higher R^2 than the M0 or M1 model.

There are plenty of studies investigating the relationship between climate factors and various infectious diseases (Lowen et al, 2007; Zuk, Rakowski & Radomski, 2009a; du Prel et al, 2009; Chan PK et al, 2009; Shaman et al, 2009; Zuk, Rakowski & Radomski, 2009a). The most intensively studied is seasonal influenza. For example, Zuk has demonstrated that spread of influenza virus depended on various temperature and relative air humidity levels (Zuk, Rakowski & Radomski, 2009a). On the other hand, there is only limited literature identified in this aspect for HFMD. Nevertheless, the fact that HFMD occurs more commonly in the temperate region and the seasonality detected by different countries suggests climate factors may have an important role in determining the activities of enteroviruses. In a Japanese study examining the relationship of weather conditions and HFMD and herpangina cases detected at sentinel paediatric clinics, it was shown that higher air temperature and humidity / vapor pressure, and lower precipitation and duration of sunshine increased

the incidence of HFMD or herpangina (Urashima, Shindo &, Okabe, 2003). This is similar to our findings although total rainfall (precipitation) and duration of sunshine or solar radiation are not significant associated with HFMD in our M2 regression model.

There are several postulations on how meteorological factors affect the viral transmissions (Abad, Pintó & Bosch, 1994; Mbithi, Springthorpe & Sattar, 1991; McGeady, Siak & Crowell, 1979). Laboratories studies have shown the stability of enteric viruses is influenced by environmental factors such as temperature and relative humidity (Abad, Pintó & Bosch, 1994; Mbithi, Springthorpe & Sattar, 1991; McGeady, Siak & Crowell, 1979). The viruses may have a more rapid decline in activity during dry seasons (Abad, Pintó & Bosch, 1994). Minhaz postulated that the viral envelop of influenza determined its persistence and transmission in various environmental conditions (Minhaz Ud-Dean, 2010). Whether the same theory applies to enteroviruses need further experimental studies. On the other hand, host behaviour may also differ in different seasons (Dowell, 2001). For example, children more often go outdoor to playgrounds during summer than winter when it is cold and windy. This may in turn facilitate transmission of enteroviruses when children come close together through respiratory droplets, open and weeping skin vesicles, or direct contact of the contaminated toys and environmental surface. In the M2 model, HFMD consultation rate is also associated with diurnal difference in daily temperature and wind speed. Higher wind speed may favour disease spreading through respiratory droplets. The exact mechanism how diurnal difference in temperature works is not well understood. This may act through diminishing immunity of the host or directly affecting the viral growth.

It should be noted that apart from climatic parameters, HFMD activity may also be influenced by other factors. Possible contributing factors include immunity of the susceptible population, prevalence of different enteroviruses circulating in the community, and public health measures implemented (Podin et al, 2006 ; Centers for Disease Control and Prevention, United States, 2000). For example, during the epidemic of SARS in 2003 in Hong Kong and pandemic influenza H1N1 in 2009, there was territory wide school closure. Such social distancing measures and intensive education on use of face mask helped diminishing the disease transmission (Lo et al, 2005). This explained why the observed HFMD rates during summer of 2009 are lower than the predicted rates in the projection analysis.

Strengths and weaknesses of current study

The trend of HFMD is a good reflection of genuine HFMD activity, as evidenced by their good correlation of the positive laboratory findings of enteroviruses (Ma et al, 2011a). On the other hand, the number of HFMD outbreaks reported to DH might subject to reporting bias since it was not mandatory and was therefore not preferred to be used in this present study. Other source of data such as the number of EV71 infection was not a good choice since the number reported each year was relatively small, making it vulnerable to random error. Besides, EV71 only accounted for a small portion among all enteroviruses causing HFMD (Please see Chapter 3). Another strength of this study was that we have obtained the longest period of available data from the SSS, covering a decade (from 2000 to 2009) in our calculation. The meteorological data obtained the Hong Kong Observatory was the official and best source information for climate parameters.

I have adjusted the correlation between various meteorological parameters before putting them in the logistic regression model. This would avoid including two variables which have high collinearity and increase the standard errors of the coefficients in a regression model.

The sentinel data used to reflect HFMD was weekly data although daily data for most meteorological parameters were available from the Hong Kong Observatory. However, this was the best available data. In this study, we did not quantify the association between various meteorological parameters and HFMD. The model will be very complicated since the effect of temperature on HFMD might be different at different humidity or other meteorological parameters. Further study might explore how HFMD activity varied under different common weather conditions.

Figure 5.1 Weekly consultation rates of HFMD detected by SSS based at GP in Hong Kong, 2000-2009

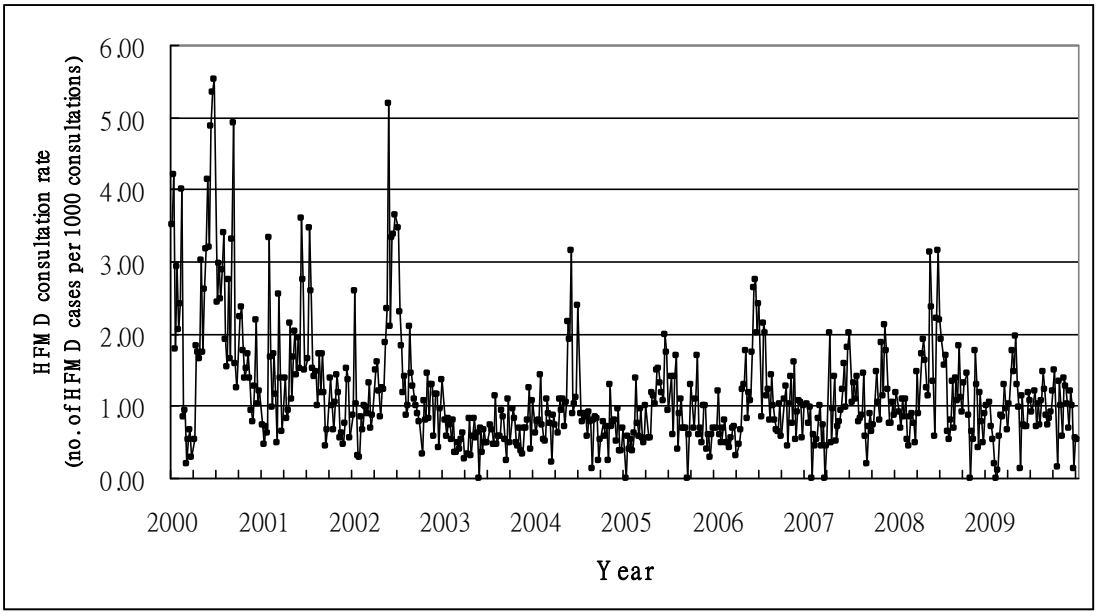


Figure 5.2 Observed and predicted weekly HFMD consultation rates, 2005-2009

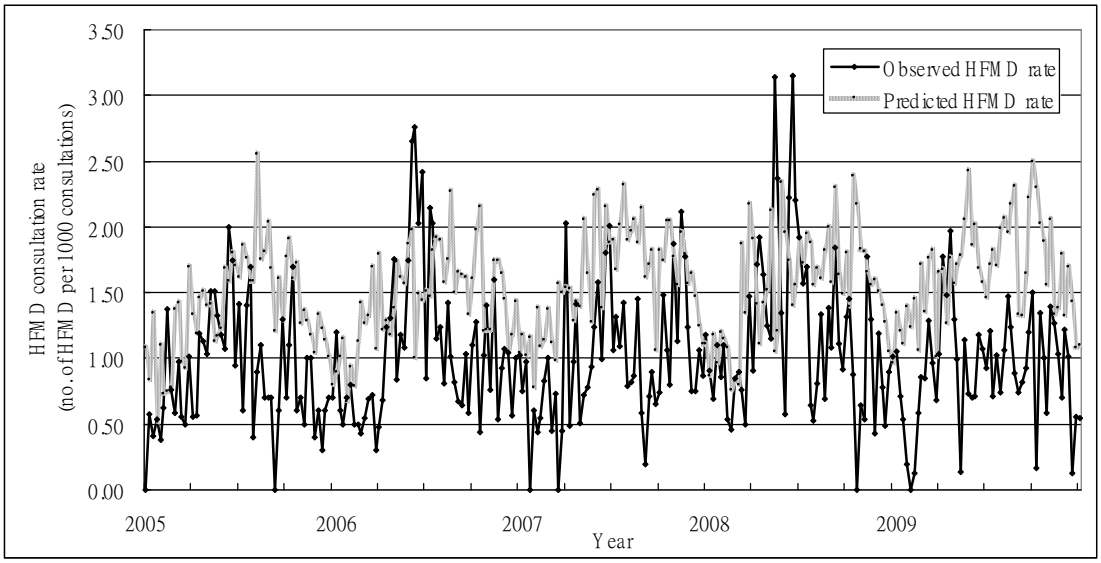
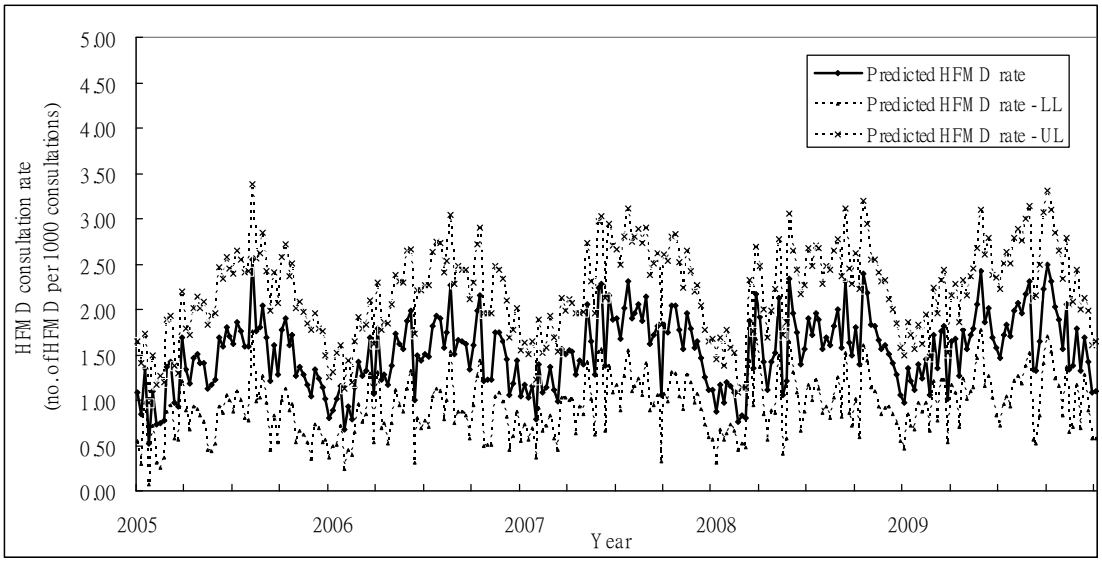
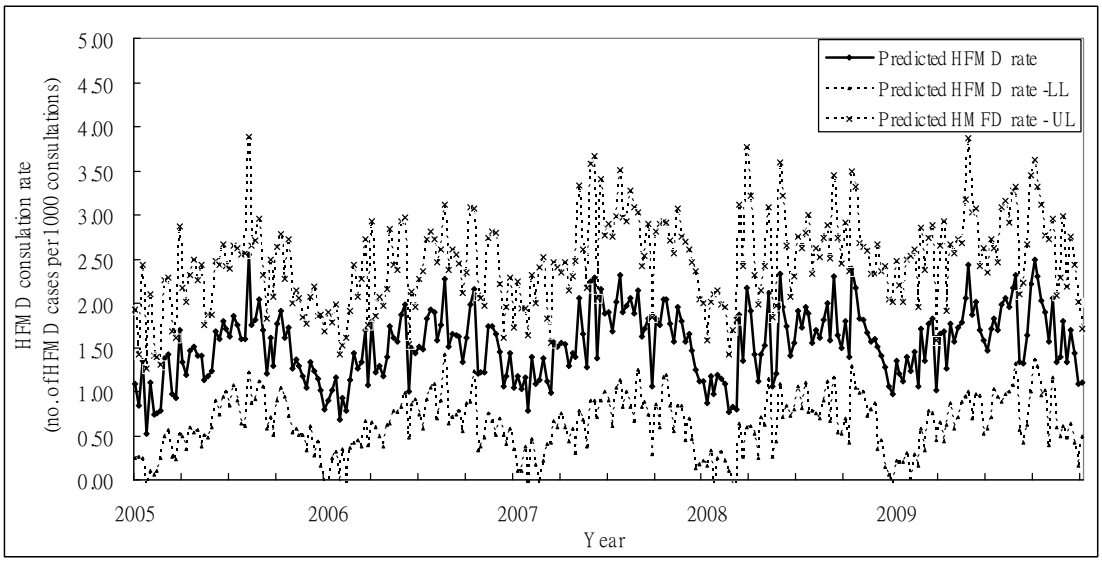


Figure 5.3 Sensitivity analysis of predicted HFMD consultation rates by varying partial correlation coefficients of mean temperature (3a), diurnal difference in temperature and relative humidity (3c) and wind speed (3d) while fixing the other meteorological parameters

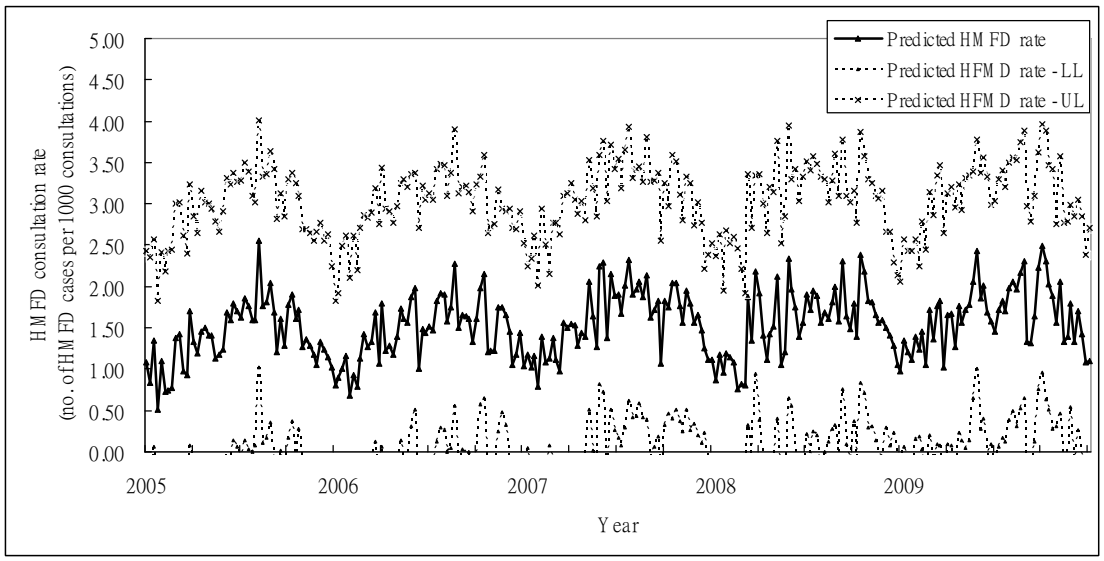
5.3a Mean Temperature



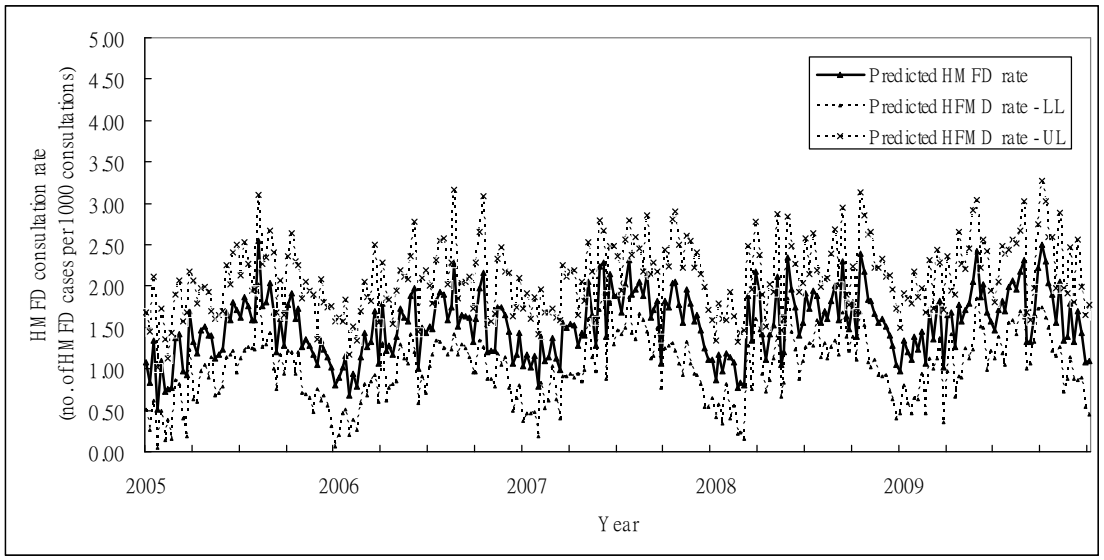
5.3b Diurnal difference in temperature



5.3c Relative humidity



5.3d Wind speed



Note: LL : lower limit

UL : upper limit

Table 5.1 Meteorological parameters used for examining the relationship with the weekly HFMD consultation rates

Meteorological parameters	Mathematical calculation	Unit
Maximum temperature	Weekly average of daily maximum temperature	°C
Mean temperature	Weekly average of daily mean temperature	°C
Minimum temperature	Weekly average of daily minimum temperature	°C
Diurnal difference in temperature	Weekly average of daily diurnal difference in temperature	°C
Dew point	Weekly average of daily dew point	°C
Atmospheric pressure	Weekly average of daily atmospheric pressure	hPa
Evaporation	Weekly average of daily evaporation	mm
Total rainfall	Sum of total rainfall measured during a week	mm
Relative humidity	Weekly average of daily relative humidity	%
Cloud amount	Weekly average of daily cloud amount	%
Total bright sunshine	Sum of total bright sunshine measured during a week	hours
Solar radiation	Weekly average of daily solar radiation	MJ/m2
Wind speed	Weekly average of daily wind speed	km/h

Table 5.2 Spearman's rank correlation between various meteorological factors and HFMD consultation rates detected by SSS, 2000-2004

Meteorological factors	Spearman's correlation coefficient	P-value
Maximum temperature*	0.261	0.000*
Mean temperature*	0.258	0.000*
Minimum temperature*	0.243	0.000*
Diurnal difference in temperature	0.139	0.024*
Dew point*	0.280	0.000*
Atmospheric pressure*	-0.334	0.000*
Evaporation*	0.212	0.001*
Total rainfall*	0.227	0.000*
Relative humidity	0.137	0.027*
Total sunshine	-0.008	0.898
Solar radiation	0.107	0.083
Wind speed	0.005	0.942

* Statistical significant ($p<0.05$)

Table 5.3 Correlation coefficient between different meteorological parameters measured from 2000-2004

Meteorological factors	Maximum temperature	Mean temperature	Minimum temperature	Diurnal difference in temperature	Dew point	Atmospheric pressure	Evaporation	Total rainfall	Relative humidity	Total sunshine	Solar radiation	Wind speed
Maximum temperature	-	*1.00**	*0.99**	0.30*	*0.94**	*-0.88**	*0.83**	0.41*	0.18*	0.35*	0.62*	-0.42*
Mean temperature	*1.00**	-	*1.00**	0.24*	*0.95**	*-0.88**	*0.82**	0.43*	0.20*	0.33*	0.58*	-0.39*
Minimum temperature	*0.99**	*1.00**	-	0.18*	*0.95**	*-0.87**	*0.80**	0.42*	0.22*	0.32*	0.58*	-0.39*
Diurnal difference in temperature	0.30*	0.24*	0.18*	-	0.12	-0.19*	0.47*	-0.03	-0.37*	0.50*	0.52*	-0.31*
Dew point	*0.94**	*0.95**	*0.95**	0.12	-	*-0.92**	0.66*	0.58*	0.46*	0.12*	0.41*	-0.40*
Atmospheric pressure	*-0.88**	*-0.88**	*-0.87**	-0.19*	*-0.92**	-	-0.59*	-0.60*	-0.44*	-0.01	-0.33*	0.33*
Evaporation	*0.83**	*0.82**	*0.80**	0.47*	0.66*	-0.59*	-	0.08	-0.23*	0.68*	*0.86**	-0.23*
Total rainfall	0.41*	0.43*	0.42*	-0.03	0.58*	-0.60*	0.08	-	0.62*	-0.45*	-0.23*	-0.07
Relative humidity	0.18*	0.20*	0.22*	-0.37*	0.46*	-0.44*	-0.23*	0.62*	-	-0.58*	-0.33*	-0.21*
Total sunshine	0.35*	0.33*	0.32*	0.50*	0.12*	-0.01	0.68*	-0.45*	-0.58*	-	*0.88**	-0.25*
Solar radiation	0.62*	0.58*	0.58*	0.52*	0.41*	-0.33*	*0.86**	-0.23*	-0.33*	*0.88**	-	-0.37*
Wind speed	-0.42*	-0.39*	-0.39*	-0.31*	-0.40*	0.33*	-0.23*	-0.07	-0.21*	-0.25*	-0.37*	-

Note:

**P* value < 0.05

Those value *italicized* are ≧0.80 indicating the two factors are highly correlated with each other

Either Pearson's or Spearman's rank correlation is used as appropriate

Table 5.4 Linear regression models using various meteorological parameters to explain HFMD consultation rates, 2000-2004

Model	R^2 for the model	P-value for the model	Meteorological parameters included in the model	Partial correlation coefficient	Lower limit	Upper limit	P-value
M0	0.079	0.011	Constant	0.291	-0.330	0.911	0.357
(No lag time)			Mean temperature	0.039	0.013	0.066	0.004*
			Total rainfall	0.002	0.000	0.004	0.011*
M1	0.062	0.000	Constant	0.053	-0.562	0.667	0.029*
(1 week lag time)			Mean temperature	0.053	0.028	0.079	0.000*
M2	0.119	0.010	Constant	-4.206	-6.481	-1.931	0.000*
(2 weeks time)			Mean temperature	0.047	0.020	0.075	0.001*
			Diurnal difference in temperature	0.395	0.187	0.603	0.000*
			Relative humidity	0.026	0.006	0.045	0.010*
			Wind speed	0.038	0.014	0.063	0.002*
M3	0.122	0.027	Constant	-4.020	-6.293	-1.746	0.001*
(3 weeks time)			Mean temperature	0.044	0.017	0.072	0.002*
			Diurnal difference in temperature	0.449	0.241	0.658	0.000*
			Relative humidity	0.022	0.003	0.042	0.027*
			Wind speed	0.037	0.013	0.061	0.003*

* Statistical significance ($p<0.05$)

Chapter 6. Study (4) -- Estimation of the basic reproduction number of Enterovirus 71 and Coxsackievirus A16 in Hand, Foot, and Mouth Disease Outbreaks

INTRODUCTION

Very often, outbreaks of HFMD occur in child care settings and schools where children have close contact. Cox A16 and EV71 can be transmitted by direct contact with nose and throat discharges and faeces of infected individuals or through aerosol droplet. However, the transmission dynamics within these outbreaks is not well understood. The basic reproductive number (R_0) has played a central role in infectious disease epidemiology as it measures the infectiousness of a pathogen in a given population. R_0 is generally defined as the average number of secondary cases infected by a typical infected person in a population that is almost fully susceptible (Heffernan, Smith & Wahl, 2005). A larger value of R_0 generally implies an increased likelihood of an infection to spread through the population causing an epidemic. The determinants of R_0 include the number of contacts per unit time, probability of transmission per contact and the duration of infectious period (Ward et al, 2009). The number of contacts per unit time and the probability of transmission per contact are often difficult to obtain in actual epidemiological field investigation although the duration of infectious period is known from literature. Estimation of R_0 has been conducted for many emerging and re-emerging infectious diseases including Severe Acute Respiratory Syndrome (SARS), avian influenza H5N1, Ebola virus, measles and *Esherichia coli* O157:H7 (Ward et al, 2009; Legrand et al, 2007; Laegreid & Keen, 2004; Mossong & Muller, 2000; Choi & Pak, 2003). However, little is

known for this important parameter for enteroviruses. To address this knowledge gap, we used a mathematical model to estimate the basic reproductive number of EV71 and Cox A16 infection in HFMD outbreaks.

METHODS

Estimating R_0 from epidemic curve

In Hong Kong, institutions such as child care centres and kindergartens are required to report suspected infectious disease outbreaks such as influenza like illness and HFMD to DH for epidemiological investigation and control. Upon notification of the outbreak, prompt investigation is conducted including case interview, site visit and laboratory investigation for causative agents accounting for the outbreaks. The management staff of the institutions will be advised on infection control measures. Children are advised not to attend schools if they develop symptoms of HFMD. An epidemic curve would be constructed for monitoring latest situation of the outbreak. An example is illustrated in Figure 5.1 showing the increase in the number of cases during the growth phase of the outbreak and it gradually decreased after initiation of public health control measures.

Different methods have been applied to estimate R_0 of other infectious diseases. These methods often involved complex mathematical equations and assumptions of hypothetical situations. I have adopted a simplified approach based on Choi and Pak's study to determine R_0 of influenza virus from outbreaks reported to DH and found

that the results (median 1.88, inter-quartile range 1.59 - 2.00, N=9) are comparable with R_0 reported from the literature (Choi & Pak, 2003, Vynnycky, Trindall & Mangtani, 2007; Jackson, Vynnycky & Mangtani, 2010; Chowell, Nishiura & Bettencourt, 2007; White et al, 2009). In this study, I used this model to estimate R_0 for EV71 and Cox A16.

Mathematical model used

I used a mathematical model to estimate R_0 of EV71 and Cox A16 infection in these outbreaks, based on the method used by Choi and Pak in predicting number of SARS cases (Choi & Pak, 2003). This model only requires R_0 and the incubation period in estimating the number of newly infected cases. I assumed each school as a closed system and the outbreak has originated from a single affected person; children in the schools were all susceptible to infection at the beginning of the outbreak; children would take sick leave after they became symptomatic. Using the epidemic curve, I estimated R_0 in the early growing phase of each outbreak. The early growing phase was defined as the period from onset of symptoms of the first case to the date when the number of newly affected cases became highest or plateau off. I estimated the value of the reproductive number by the following formula:

$$\sum N_{t-i} = 1 + R_0 + R_0^2 + R_0^3 + \cdots + R_0^t$$

where N_{t-i} is the predicted number of incident cases on day $t-i$, and t is the time expressed in the number of incubation periods. After one incubation period, one case would spread to R_0 cases and the cumulative number of infected cases would be $1 + R_0$. Similarly, after two incubation periods, the cumulative number of infected cases

would be $1 + R_0 + R_0^2$. For example, if R_0 is equal to 3 and incubation period is 5 days, there will be 1 + 3 cases during the first sixth days (1 + 5 days) of the outbreak while in the first 11^{th} days (1 + 5 + 5 days), there will be $1+ 3 + 3^2$ cases. The usual incubation period for HFMD is three to five days, with longest period of 7 days (Heymann D., 2004; Wong S.S. et al, 2010). I assumed the incubation period to be 5 days in the present analysis. R_0 can then be estimated from the cumulative number of cases at the initial growth phase of the outbreak as determined by the epidemic curve. Alternatively, the formula is expressed as:

$$\sum N_{t \cdot i} = (R_0^{t+1} - 1) / (R_0 - 1)$$

where t = number of days of the initial growth phase of the outbreak divided by incubation period.

Statistical analysis

I first identified all HFMD outbreaks reported to the DH from the electronic records between 2004 to 2009, which were confirmed to be associated with EV71 or Cox A16 by laboratory tests using viral culture or polymerase chain reaction. I recruited outbreaks which involved more than 15 persons affected during the whole investigation period. This was because small outbreaks affecting only a few persons would result in large random error in estimation of R_0. Nevertheless, to address whether the selection of outbreaks involving 15 persons would lead to any bias, the estimated R_0 was plotted against the number of persons affected and regression analysis was used to test for any association. I also examined for any association between estimated R_0 and setting of outbreaks (whether the outbreak occurred in

child care centre or kindergarten) and size of the institution (number of persons exposed). The estimated R_0 for EV71 was compared with Cox A16 using Mann-Whitney U test to examine for statistical difference. In addition, I conducted sensitivity analysis by varying the incubation period from three days to seven days to examine the corresponding change in R_0. Statistical Package for the Social Sciences, version 14.0, Chicago was used for all statistical analysis.

RESULTS

There were 34 outbreaks identified, 27 outbreaks due to CoxA16 and seven outbreaks due to EV71 (Table 6.1). Among these 34 outbreaks, 20 occurred in child care centres while 14 occurred in kindergartens, with a median size of about 120 persons. The attack rates of these outbreaks ranged from 5.7% to 38.8% (median 19.3%) and lasted for about three weeks. Majority of the patients recovered without complication. It was noted that the attack rates of the outbreaks due to CoxA16 and EV71 were similar, but the outbreaks due to EV71 was in general shorter than those caused by CoxA16 (median: 17 days vs 24 days), although the difference was not shown to be statistically significant by Mann-Whitney U test. There was also no statistical difference in other key characteristics (number of persons exposed and proportion of persons hospitalized) between the outbreaks due to CoxA16 and EV71.

The median R_0 of EV71 was estimated to be 5.48 with an inter-quartile range of 4.20 – 6.51 while that of CoxA16 was 2.50 with an inter-quartile range of 1.96 – 3.67 (Figure 6.2). The estimated R_0 of EV71 was significantly higher than that of CoxA16

by Mann-Whitney U test, p=0.024. As illustrated in Figure 6.3 and Figure 6.4, the R_0 estimated was not associated with outbreak settings (whether in child care centre or kindergarten) and sizes of the institution (number of persons exposed). In addition, the estimated R_0 was not associated with the number of persons affected in the outbreak selected, as shown by the regression analysis in Figure 6.5. In the sensitivity analysis, R_0 for both EV71 and CoxA16 increased if I assumed a longer incubation period. In the whole range of incubation periods tested, R_0 of EV71 was consistently higher than that of CoxA16 by Mann-Whitney U test, with all p-values less than or equal to 0.025 (Figure 6.6).

DISCUSSION

Interpretation of major findings and relationship with published literature

To my best knowledge, this is the first report of R_0 for EV71 and CoxA16 in the literature. The present estimation for R_0 of EV71 was 5.48, more than double that (2.50) of Cox A16 using data of outbreaks of HFMD. This infers that for every child acquiring EV71 infection, they would further infect about 5 other children at the beginning of these outbreaks in kindergarten and child care centre, while children acquiring CoxA16 infection would infect only two more persons. This finding helps us better understand the transmission dynamics of HFMD outbreaks and formulate control measures in these settings. For example, in Singapore, childcare centres and kindergartens with more than 16 HFMD cases or an attack rate greater than 23%, and a transmission period of more than 24 days will be mandatorily closed for 10 days (Singapore Ministry of Health, 2010). The present knowledge of R_0 would assist predicting the epidemic curve of an outbreak and in turn help better decision making

for class suspension or even school closure.

There is no other report identified for comparing our estimates of R_0 with others. The median R_0 for EV71 is 5.48 while that of CoxA16 is 2.50. This is considered as moderately infectious when compared to other infectious diseases (Ward et al, 2009; Legrand et al, 2007; Laegreid & Keen, 2004; Mossong & Muller, 2000; Choi & Park, 2003). R_0 for more infectious agents such as measles, an airborne virus, was estimated to be 6.2 to 7.7 while that for norovirus (spread through aerosol) was reported to be as high as 14.05 (Mossong & Muller, 2000; Heijne et al, 2009). It is interesting to note that the estimated range of R_0 of enteroviruses is similar to that of many other viruses spread by respiratory droplets such as influenza (R_0=2.0 to 3.0) and SARS (R_0=2.7) (Vynnycky et al, 2007; Jackson et al, 2010; Chowell et al, 2007; White et al, 2009). Although HFMD has been well promulgated as a disease spread by faecal-oral route, it is postulated that respiratory transmission may contribute more to the epidemic spread during outbreak than faecal-oral transmission in developed areas (Jackson, Vynnycky & Mangtani, 2010). In fact, it has been demonstrated from laboratory that oropharyngeal secretions contained a higher viral load but a shorter duration of shedding than faeces (Wang J.R. et al, 2000; Ooi M.H. et al, 2007a). Both factors would result in a higher value of R_0 since they would increase the number of people affected within a short period of time. Furthermore, it is not uncommon for patients with enterovirus infection to present with respiratory tract symptoms. Chang et al had reviewed that 24% to 40% of patients infected with EV71 and CoxA16 also had respiratory symptoms of cough or rhinorrhea while only 6% to 10% had diarrhea (Chang et al, 1999a). In a recent review of HFMD outbreak in Taiwan in 2008, Chen et al also found similar results for EV71 and coxsackievirus A2 (Chen S.P. et al,

2010). Hence, preventive measures should not only focus on enteric precaution such as hand washing, but also need to emphasize the importance of measures against droplet transmission such as wearing face mask if patients have respiratory symptoms.

It is well known that EV71 is more likely than CoxA16 to cause severe complications such as meningitis, encephalitis and pulmonary edema although both viruses are Picornaviruidae and are closely related genetically under the species human enterovirus A. Previous efforts in identifying the genetic composition to account for their difference in virulence did not have promising results (Bible et al, 2007). The present findings add that EV71 is more infectious than CoxA16 and hence is more likely to cause large outbreaks. The shorter duration of outbreak noted in the cases caused by EV71 (median 17 days for EV71 vs 24 days for CoxA16) is also compatible with this finding. EV71 spreads more efficiently among children and requires less time to reach the same attack rate when compared to CoxA16. The exact mechanism on why and how EV71 being more infectious is not known. One postulation is that a larger proportion of the children are immune to CoxA16 compared to EV71. This is supported by the finding that CoxA16 is on average 5-fold more commonly identified than EV71 in HFMD outbreaks (Ma et al, 2010b). Further studies are required to explore for underlying reasons such as difference in molecular structure or gene functions. Nonetheless, these results reinforce the importance for laboratory surveillance of EV71 because of its potential public health implications and that EV71 has been demonstrated to have cyclical epidemics every 3 to 4 years in Hong Kong (Ma et al, 2010b) (Please also see Chapter 4) .

Strengthen and weakness of the study

This is the first report of R_0, an important epidemiological parameters, for understanding the transmission of EV71 and CoxA16 in the literature. The model I used has been verified by calculating the R_0 of influenza and I found that the result was comparable to that reported in the literature. The present approach has adopted a simplified model to estimate R_0 using field data. Previous study only used simulated data to study determining factors for disease transmission in a compartmental SEIR model without actual calculation of R_0 (Nandita & Nilimesh, 2010). Alternative method to estimate R_0 based on population approach is less feasible because many of the parameters used in other methods are not available for HFMD. For example, population-based data for number of cases of HFMD is not available because HFMD is not a statutory notifiable disease in Hong Kong. Besides, information on the proportion of susceptible population is also limited due to lack of seroprevalence study. The present model is a simplified method which does not require these parameters. Using actual field data from HFMD outbreaks in Hong Kong also increased the validity of these results.

However, there are limitations of this study that are worth noticing. First, I have used outbreak data to estimate of R_0. Using outbreak data could potentially lead to heavily biased and unreliable estimates of R_0 due to selection bias in identification and investigation of outbreaks. For example, high transmissibility may have led to an unusually large number of infections within a short period, leading to an outbreak being investigated and analyzed. Conversely, lower transmissibility in another setting would not have led to outbreaks, and that situation would not be reported and

analyzed. However, it is noted in the letters issued to all child care centres and schools by Department of Health, the institutions are urged to report any suspected outbreak of HFMD, regardless of their size: "*If you suspect a HFMD outbreak (e.g. two or more children in the same class or having same group activities and develop HFMD within a period of 7 days), please inform the Central Notification Office of CHP as early as possible…*" (Centre for Health Protection, 2008). An outbreak of HFMD is defined as two or more children in the same class or having same group activities and develop HFMD within a period of 7 days. Such a low "threshold" of the outbreak definition would essentially require all schools to report any intra-school transmissions of HFMD if it really happens, regardless whether it leads to a large number of cases within a short period of time or only two cases within the maximum possible incubation period. In order words, they are required to report HFMD outbreaks to the Health Authority regardless of the degree of transmissibility of the enterovirus. In addition, I also examined whether there was any bias to include large outbreaks and lead to over-estimation of R_0. The results in Figure 6.5 showed that there was no statistical association between size of outbreaks and the estimated R_0.

Second limitation is that I assumed a close compartment within the child care centres or kindergarten and there was only one index case at initial phase. If the children were infected by family members instead of members of the institution, or if the school outbreaks originated from more than one child, the estimated R_0 would be higher than the actual one. Another limitation was the small number of outbreaks caused by EV71, as reflected by the wide inter-quartile range. Increasing the number of outbreaks recruited could probably have a higher precision on the estimates. Besides, I did not investigate the effect of control measures on estimation of R_0. The public health

control measures implemented after notification of the outbreak would probably reduce the number of cases and hence R_0. Nevertheless, it was noted that for most (64.7%) of the outbreaks, the peak of the epidemic curve occurred not later than 7 days after notification when the expected effect of control measures started to appear. Therefore, the effect of underestimation of R_0 caused by public health measures would not be large.

Figure 6.1 Epidemic curve of a hand, foot, mouth disease outbreak reported in Hong Kong

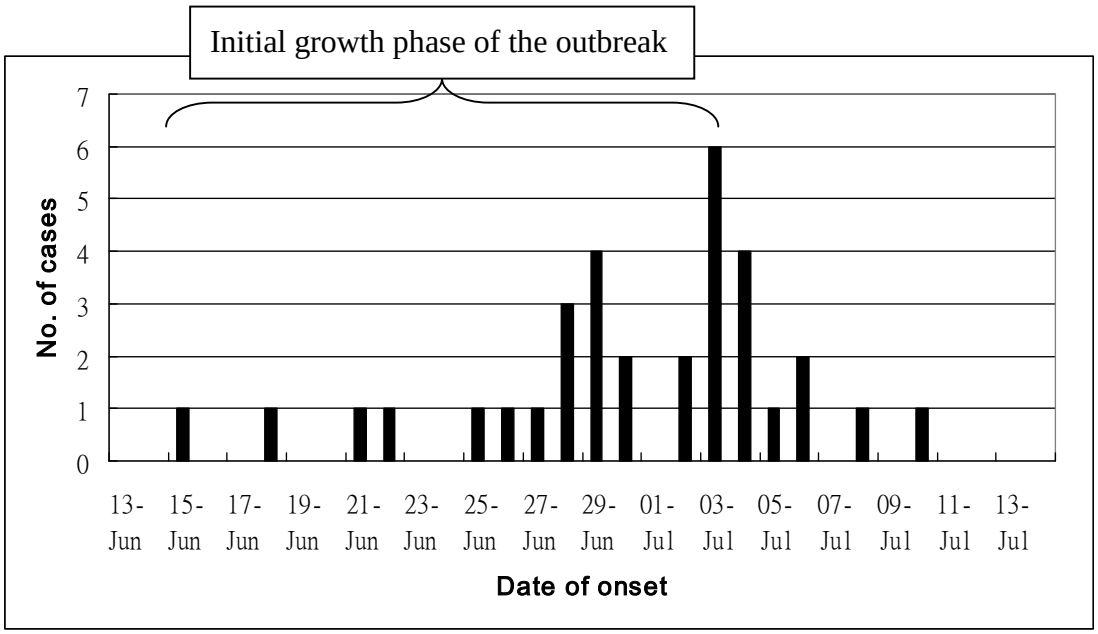
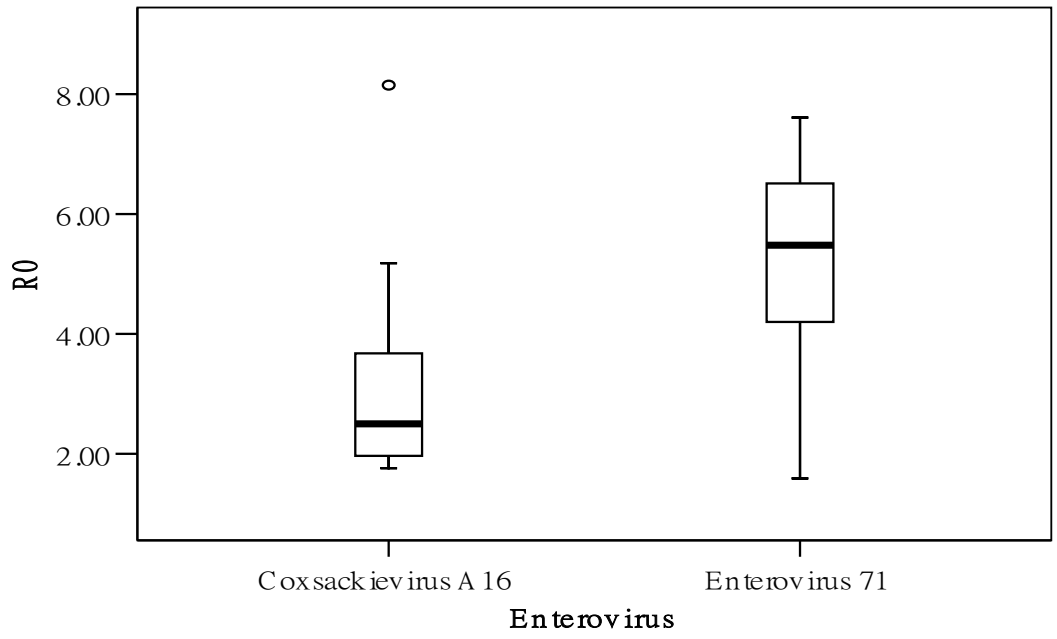
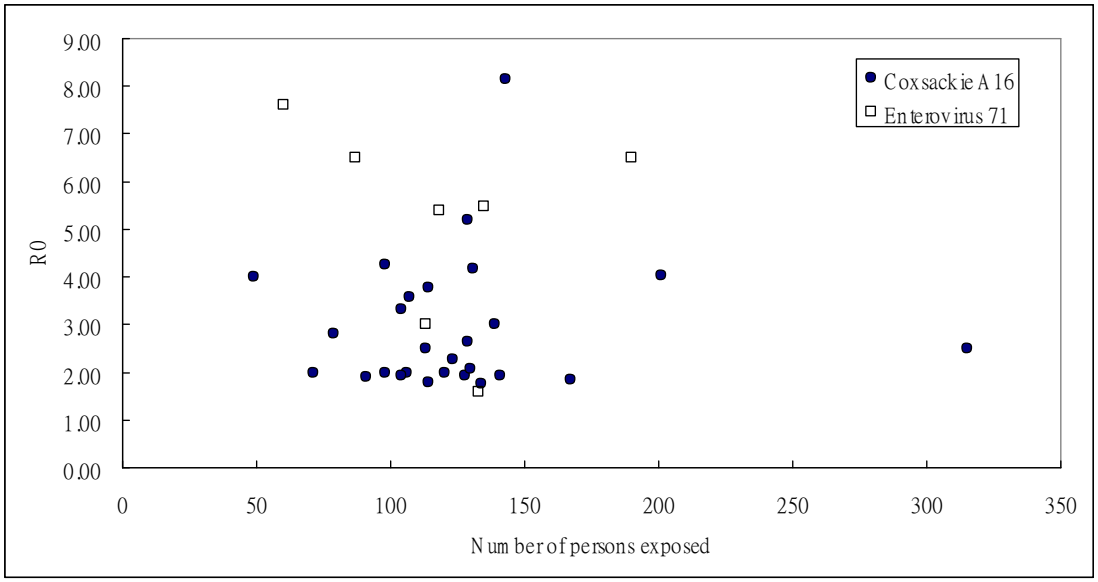


Figure 6.2 Estimated R_0 of EV71 and CoxA16 in HFMD outbreaks



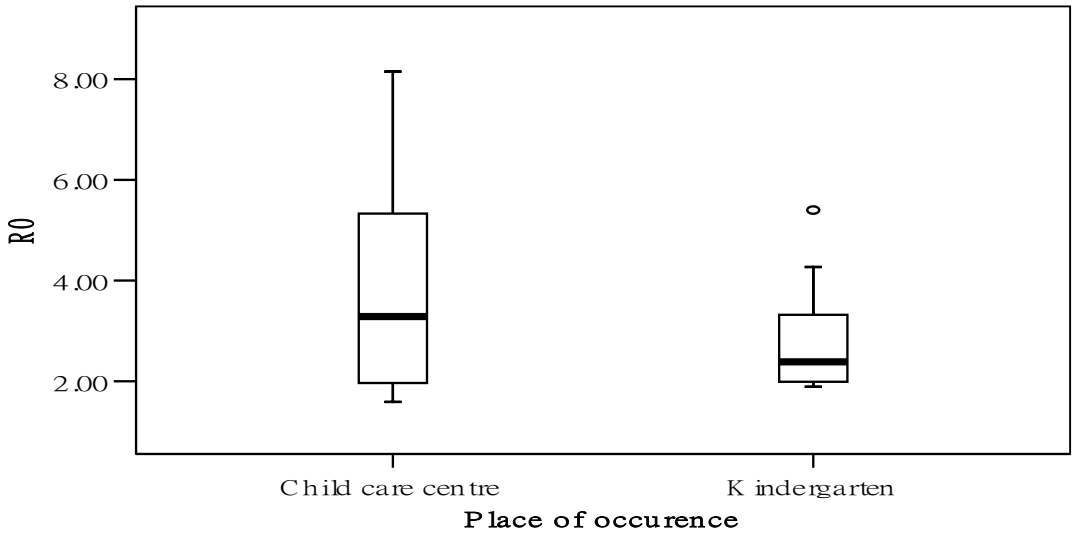
The estimated R_0 of the EV71 was significantly higher than that of CoxA16 by Mann-Whitney U test, p=0.024. Circle for CoxA16 indicated an outlier.

Figure 6.3 Scatter plot of estimated R_0 against the number of persons exposed in HFMD outbreaks



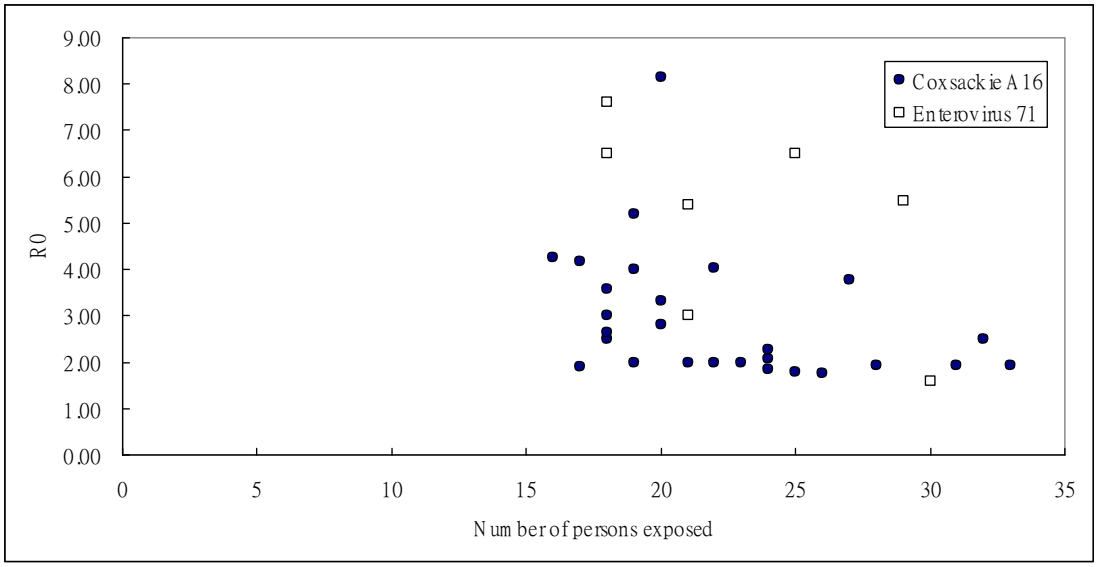
Estimated R_0 was not associated with number of persons exposed by liner regression test, p=0.791

Figure 6.4 Estimated R_0 against different settings of HFMD outbreaks



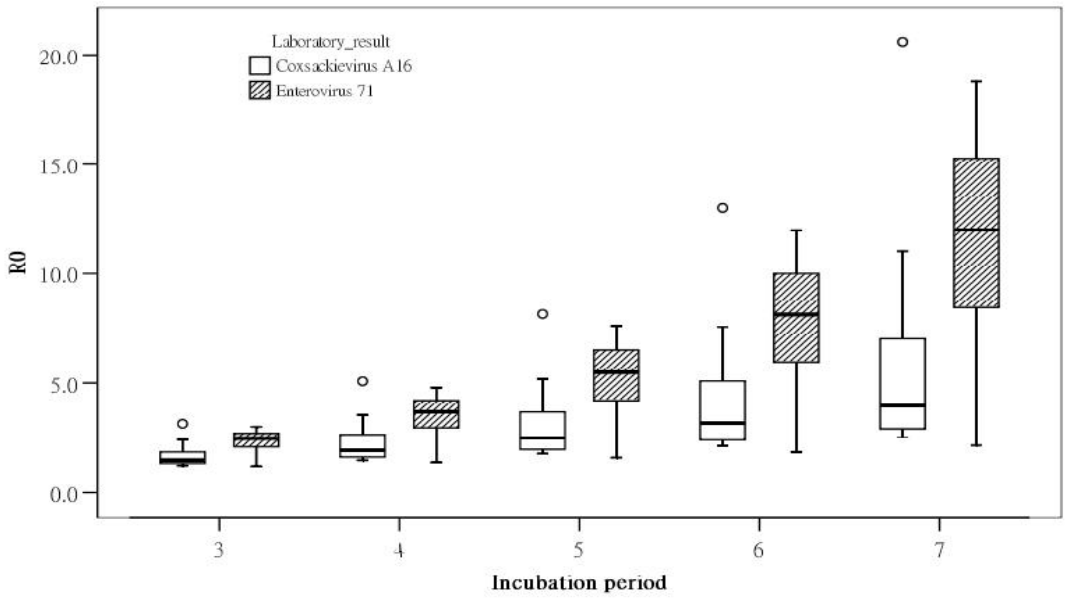
The estimated R_0 were not statistically different in different outbreak settings by Mann-Whitney U test, p=0.31. Circle for kindergarten indicated an outlier.

Figure 6.5 Scatter plot of estimated R_0 against outbreak size (number of persons affected) in HFMD outbreaks



Estimated R_0 was not associated with outbreak size by liner regression test, p=0.062

Figure 6.6 Sensitivity analysis of estimated R_0 according to different incubation period



In all scenarios, R_0 of EV71 was significantly higher than that of Cox16 by Mann-Whitney U test, all $p \leqq 0.025$. Circle for CoxA16 indicated an outlier.

Table 6.1 Characteristics of HFMD outbreaks due to CoxA16 and EV71

	Overall	Coxsackievirus A16	Enterovirus 71	p-value*
Number of outbreaks recruited (child care centre (CCC) : kindergarten (KG))	34 (20 CCC: 14 KG)	27 (15 CCC: 12 KG)	7 (5 CCC: 2KG)	0.672
Number of persons exposed in each outbreak, range (median)	49 – 315 (119)	49 – 315 (120)	60 – 190 (118)	0.966
Attack rate in each outbreak, range (median)	5.7% - 38.8% (19.3%)	5.7% - 38.8% (19.2%)	13.2% - 30.0% (20.7%)	0.608
Duration of outbreak in days, range (median)	11 – 40 (20.5)	11 – 40 (24.0)	12 – 31 (17.0)	0.130
Proportion of persons hospitalized, range (median)	0% - 17.6% (0%)	0% - 17.6% (0%)	0% - 6.9% (0%)	0.144

*All factors were not statistically different by Fisher exact test or Mann-Whitney U test

Chapter 7. Study (5) –Impact of SARS and Pandemic Influenza H1N1 on Transmission of Hand, Foot, and Mouth Disease in Hong Kong

INTRODUCTION

Since there is currently no effective vaccine or chemoprophylaxis for controlling HFMD, public health interventions rest almost solely on non-pharmacological measures. These include heightening awareness of the disease, promoting personal hygiene such as covering nose and mouth when cough or sneezing, hand washing, cleansing and disinfection, social distancing and school closure. Such public health measures are often implemented before or during the peak season of HFMD. Nevertheless, there have been few studies to evaluate them.

Apart from fecal-oral route and direct contact with open skin vesicles, enteroviruses can also be transmitted through droplets since it is not uncommon for patients with HFMD to have respiratory symptoms. Besides, indirect transmission through contaminated objects can also occur as enteroviruses can survive as fomites at room temperature.

In Hong Kong, sustained and intensive public health interventions were implemented during the Severe Acute Respiratory Syndrome (SARS) outbreak in 2003 and pandemic influenza H1N1 in 2009, featuring territory-wide school closure and massive hygiene campaign on hand washing, wearing mask and disinfection. Whiling knowing HFMD has similar routes of transmission with SARS and influenza H1N1, I take the opportunity to evaluate the impact of these measures on HFMD transmission

in the community. I compared the observed HFMD consultation rates and the projected rates in defined periods of 2003 and 2009 during which territory-wide public health interventions against SARS and pandemic influenza H1N1 were implemented.

METHODS

I used the sentinel surveillance data collected from a network of 40 general practitioners to assess the community activity of HFMD from January 2001 to June 2010. The GP who participated in the surveillance system reported the weekly number of HFMD cases to the DH. The consultation rate of HFMD was calculated by dividing the total number of HFMD by the total number of consultations seen by the sentinel doctors. I compared the observed HFMD consultation rates and the projected rates in defined periods of 2003 and 2009 during which territory-wide public health interventions against SARS and pandemic influenza H1N1 (2009) were implemented. The period in 2003 was defined to span from week 7 to week 31 during which Hong Kong experienced the SARS epidemic. In 2009, the period was defined to span from week 17 when outbreaks of the pandemic influenza H1N1 was first reported in Mexico, to week 40 when schools in Hong Kong re-opened after the summer break.

I first found the best-fitted model in projecting weekly HFMD rates by decomposition-regression analysis using data collected from January 2001 to June 2010, excluding the data collected in the two defined periods in 2003 and 2009. I took into account of any long term trend, cyclical and seasonal patterns of HFMD in

projecting the HFMD consultation rates. I included variables into the regression model to test if there was any linear trend from January 2001 to June 2010, 2-year or 3-year cycles, and seasonal pattern. Statistics Analysis System software was used to determine the statistical significance of all parameters inputted and stepwise regression was adopted in parameters selection. The percentage reduction in HFMD rates was then computed by calculating the percentage difference in the areas under curve of the observed rates and the projected rates.

RESULTS

The best-fitted model was identified by the regression analysis with adjusted R^2 value of 0.22. The observed and the projected HFMD rates from 2001 to 2010 June were illustrated in Figure 7.1, which showed that the two rates matched quite well with each other. HFMD consultations rates showed a statistically significant two-year cyclical pattern, $p<0.05$. The three-year cyclical pattern was not significantly associated with HFMD consultation rates. Seasonal effect was also found to be statistical significant for explaining the HFMD rates with higher activity detected in summer months. There was no significant linear trend in the study period. The final regression model constructed was:

$$R(t)= 1.11549 – 0.42384 \cos(2\pi *m/12) – 0.10447 \sin(2\pi *m/24)$$

where R(t): projected HFMD rate at week t

t: the number of week counting from week 1 of 2001

m: the number of month counting from January of 2001 covering week t

There was a reduction of 57.2% (95% C.I.:53.0-60.7%) in HFMD consultation rates during the SARS period in 2003 and a reduction of 26.7% (95% C.I.:19.5-32.7%) during the pandemic influenza H1N1 period in 2009. In both 2003 and 2009, the projected HFMD rates were lower than the observed rates during the whole period of school closure (Figure 7.2 and 7.3).

When I compared the projected and the observed HFMD rates till the end of these two years, it was noted that the projected rates was still lower than the observed rates in 2003 beyond week 31 until almost the end of the year. On the contrary, in 2009, the observed HFMD consultation rates become comparable to that of the projected rates in August, before the end of the defined intervention period (i.e., re-opening of schools).

DISCUSSION

Interpretation of major findings and relationship with published literature

HFMD can be spread through multiple routes. The main mode of transmission is by the faecal-oral route and respiratory droplets but direct contact with open and weeping skin vesicles or contaminated objects may also transmit the viruses. The role of environmental factors in causing enterovirus infection is controversial. The relative importance in these roles for viral transmission is not exactly known. SARS and influenza H1N1 shared similar route of transmission through respiratory droplets. All these viruses can be found in oropharyngeal secretions of affected patients. The

territory-wide campaign on public health measures during these two pandemic periods have far reaching implications on the transmission of HFMD, although these measures were not initially implemented to target enteroviruses. Wearing a facemask may reduce the transmission of enteroviruses through droplet route. Proper and frequent hand hygiene would definitely reduce faecal-oral transmission of various viruses. Social distancing measures like class suspension and school closure would halt the transmission through direct contact from affected children to non-infected ones. Using a statistical model and taking into account long term trend, cyclical and seasonal effects, the present study found and quantified the reduction in HFMD activities during the two defined periods, a possible summation of all these public health interventions. There are few comparable studies in the literature. A reduction in the number of HFMD cases was reported in Singapore during a HFMD epidemic in 2000 following implementation of similar public health measures, including national wide closure of all preschools from October 1 to 15 (Chan K.P. et al, 2003). However, no measurement was made to quantify the effect of these interventions.

The percentage reduction (57.2%) in HFMD measured in SARS period in 2003 was nearly double that measured during pandemic influenza H1N1 in 2009 (26.7%). In addition, it was worth noting that a sustained effect of reduced transmission was observed in 2003 till end of the year, while the effect gradually dampened around August in 2009 despite persistence of public health interventions. This might be explained by the different risk perception towards the two diseases which in turn affected people's preventive behaviour (de Zwart et al, 2009). SARS carried a high mortality rate and was first detected in neighboring Guangdong province in Mainland China. In contrast, pandemic influenza H1N1 (2009) started much further away in

Mexico, and more scientific evidence emerged since August 2009 suggesting that pandemic influenza H1N1 mostly caused a mild illness and the mortality was lower than that estimated at the initial phase (Wilson & Baker, 2009; WHO, 2009a; Centers for Disease Control and Prevention, United States, 2009). Certain cognitive and emotional factors such as perceived fatality and perceived vaccine availability would affect peoples' preventive behaviour including wearing facemask and hand washing at the initial phase of pandemic influenza H1N1 in Hong Kong (Lau J.T. et al, 2010). It would be useful to track how these perception changed as the pandemic evolved as so to better formulate sustained preventive strategies.

School closure was often considered a key component in controlling outbreaks of HFMD but there was limited scientific evidence from the literature on its sole impact on HFMD. Instead, school closure has been investigated as a non-pharmacological intervention to halt influenza pandemic. Using sentinel data of influenza, the effect of school closure has been quantified to reduce the cumulative number of cases by 13-17% during a pandemic (Cauchemez et al, 2008). In another study, school closure was estimated to reduce 28% of physicians' visits for respiratory infection among children (Heymann A. et al, 2004). Although the study cannot segregate the effect of school closure from other public health measures, the results did provide supportive evidence since the projected HFMD rates were lower than the observed rates during the whole period of school closure in both years.

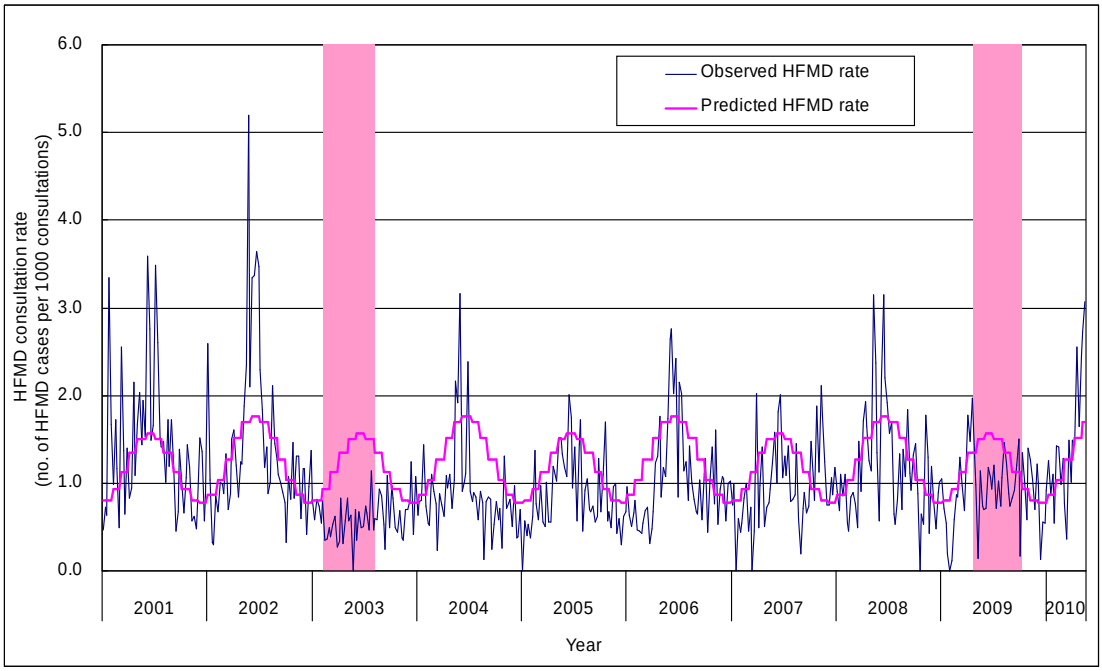
Strengthen and weakness of the study

It is often difficult to conduct evaluation of public health prevention measures since they are implemented territory-wide and there is no appropriate control group or population. I have successfully identified the study period during 2003 and 2009 when epidemic of SARS and pandemic influenza H1N1 (2009) occurred and public health control measures were conducted against these infections. The baseline data outside these two periods were used as control. Such approach was also adopted in a similar study to reduction of respiratory infections during SARS period in Hong Kong (Lo et al, 2005).

This study has several limitations. The observed reduction in HFMD may be due to the effects of multiple public health measures, including territory-wide school closure and other non-pharmacological strategies, but their individual contributions cannot be separately measured or inferred. The study did not look at the effect of children spreading HFMD in non-school settings when schools were closed. It has been shown that intra-familial transmission of enterovirus 71 was as high as 84% among siblings and 41% from children to their parents (Chang et al, 2004a). In projecting the HFMD consultation rates, I have taken into consideration of the long term trend, cyclical and seasonal pattern, which are considered as the major determinants of HFMD in our regression model. Nonetheless, there may exist some other factors which also contribute to the trend of HFMD. For instance, a Japanese study has shown that climate factors such as air temperature, humidity, precipitation and duration of sunshine also affected the incidence of HFMD or herpangina (Urashima, Shindo & Okabe, 2003). Further research may need to explore other possible factors

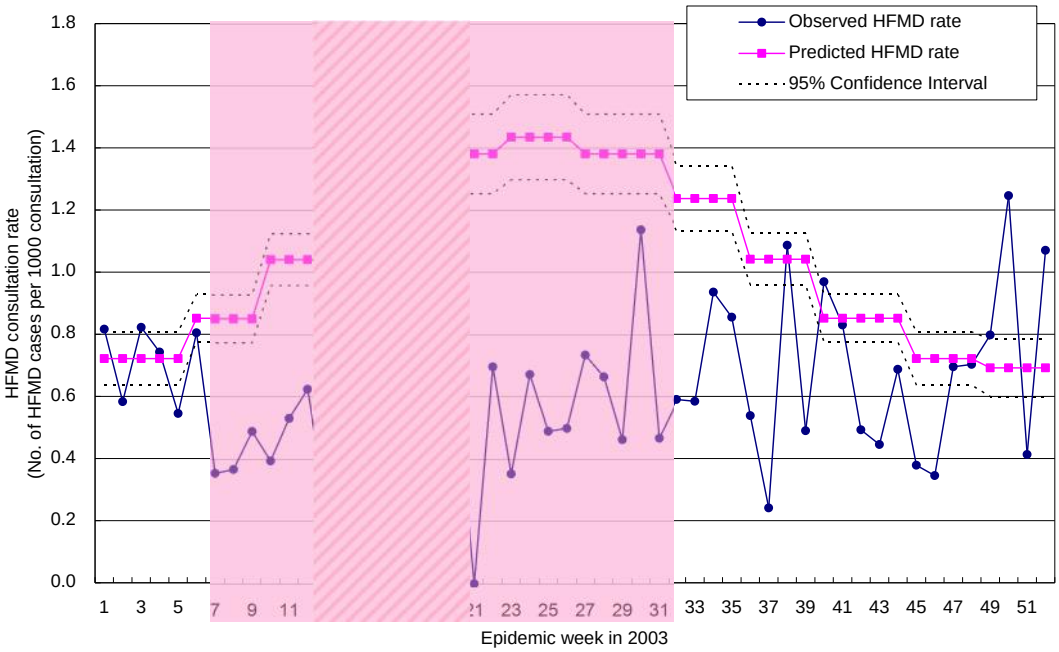
in predicting trends of HFMD.

Figure 7.1 Projected and observed HFMD consultation rates reported by SSS from January 2001 to June 2010



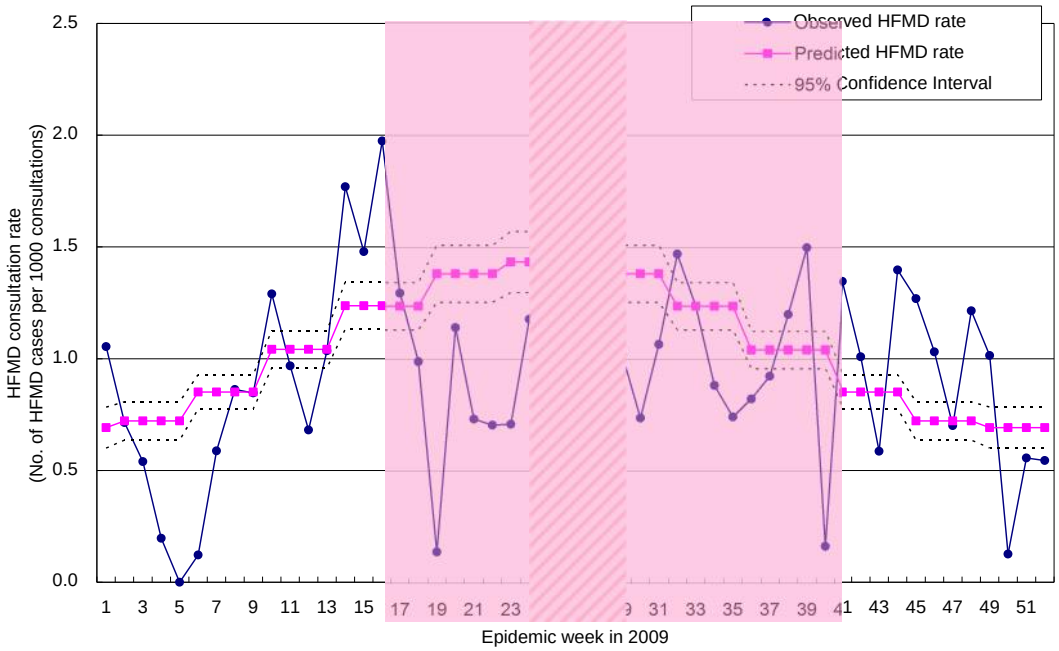
The projected rates match well with the observed ones except in the shaded periods, i.e. the periods when enhanced public health measures were implemented.

Figure 7.2 Projected and observed HFMD consultation rates in 2003



The shaded area indicated the period when territory-wide public health measures were enhanced during week 7 to week 31 in 2003. Schools were closed during week 13 to week 20 in 2003.

Figure 7.3 Projected and observed HFMD consultation rates in 2009



The shaded area indicated the period when territory-wide public health measures were enhanced during week 17 to week 40 in 2009. Schools were closed during week 24 to week 29 in 2009.

Chapter 8. Conclusion

In summary, through this thesis, I have provided new knowledge and addressed the knowledge gap identified from the current medical literature. In study (1), I have provided a clear account of the local features of epidemiology of HFMD in the past decade. It has identified the cyclical pattern of EV71 infection with specific years of high activity recorded. It also documented the summer peak of Hong Kong for this infection and reported a recent trend of winter peak which was not well known from the current literature. I reported a rising trend of young adolescents being affected, meaning that clinicians should not forget about this differential diagnosis since complications can still happen in this group.

Surveillance for HFMD is prudent because there is no effective chemoprophylaxis or vaccine available (McMinn, 2002; Wang S.M. & Liu, 2009; Lee M.S. & Chang, 2010; Zhang D., Lu & Lu, 2010; Xu J., et al, 2010). Social distancing or avoiding contact with HFMD case, maintaining good personal hygiene, and disinfection of the environment are probably the most effective ways for prevention. The surveillance findings in study (1) may guide public health actions, e.g. issue early warning for both summer peak from May to July, and winter peak from October to December. Public health education through press release, media interview and letter-to-institutions can alert parents and school managers on how to prevent the infection.

Although the dominant susceptible population is still under 5 years old, which is consistent with findings of study (1), I found that more teenage children were also affected by HFMD in recent years. Physicians should be given this information to assist them to make correct diagnosis, especially during the peak seasons of the disease. Besides, prevention measures by public health authorities should also reach primary and secondary schools instead of child care centre only.

The cyclical trend of EV71 detected has far reaching implication. The proportion of EV71 diagnosed by Public Health Laboratory Centre was particularly high in the year 2001, 2004 and 2008 (Table 3.1). In these years, the HFMD activities were also higher than other years in the past decade. More importantly, our study also showed that the hospitalization rate of HFMD was higher when EV71 was more active in that year. The health care system in Hong Kong should be prepared for the next high epidemic year to come after waiting for a cycle 3 to 4 years.

Study (2) has provided detailed epidemiological information of EV71 infection in Hong Kong from 1998 to 2008. This does not only include the clinical presentation of this important infection, but also the complications, cyclical trends and molecular epidemiology. In particular, the complication rate and case fatality rate provide basis for future analysis if large epidemic occur again in the future. The phylogenetic analysis would be very useful to track the circulation of various strains, especially for those uncommon ones like C1-2 and B3-5.

In 2008, the activity of HFMD was particularly high and both public and media were scared about the infection. Scientific data were required to assess the real situation in order to convey the rational risk perception. Study (2) demonstrates the appropriate approach in comparing the complication rate, case fatality rate, and molecular strain of EV71 in 2008 with that of the baseline information collected from 1998 to 2007. This helps reassuring the public to avoid unnecessary panic.

Although the clinical presentation and predominant strain of EV71 in Hong Kong in 2008 were similar to that in past years, the cyclical high activity has caused significant public health and social implications. Currently there is no effective chemoprophylaxis or vaccine for EV71. While we are waiting for an effective vaccine to be developed, early case detection and prevention of secondary spread appear to be the most effective public health measures for controlling EV71 infection (McMinn, 2002). To strengthen public health surveillance and control, EV71 infection was made a statutory notifiable disease in Hong Kong since March 2009. Doctors are required to report cases of EV71 by law to DH for further investigation and control. It is important to recognize that some EV71 infection did not have prominent HFMD presentation but instead respiratory symptoms. In fact, this study documents the first fatal case of EV71 infection due to interstitial pneumonitis in Hong Kong. This helps alerting doctors that EV71 should be one of the differential diagnoses when dealing with paediatric patients during peak season of HFMD.

In study (3), I provide direct evidence on how HFMD activity is affected by various meteorological factors and report their correlation coefficients. By comparing these

correlation coefficients, I demonstrated the relative importance of these climate factors on activity of HFMD. This is also echoed in the sensitivity analysis, which is rarely seen in other similar studies. I take into account the possible co-relationship among the different meteorological factors and adjust them in the final model. The models also examined the possible lag time on effect of HFMD by these meteorological factors. This information is also new to the current understanding of epidemiology of HFMD.

Climate change has been considered as the biggest global health threat of the 21st century (Costello et al, 2009). There is much concern on the potential impact of global warming on infectious diseases (McMichael, Woodruff & Hales, 2006). In a model constructed in Japan, the authors simulated the impact of global warming on the incidence of HFMD by varying vapor pressure, temperature and relative humidity parameters and found that the disease activities might increase by 7% to 14% (Urashima, Shindo & Okabe, 2003). Based on projections by climate models, the Intergovernmental Panel on Climate Change in their Fourth Assessment Report indicated that the global average surface temperature would rise by 1.1 to 6.4℃ by the end of the 21st century (Parry et al, 2007). In a forecast conducted by the Hong Kong Observatory, it was estimated that by 2090-2099, relative to the 1961-1990, the annual mean temperature in Hong Kong would rise by 1.7°C to 5.6°C, with an ensemble mean of 3.5°C (Leung, Wu & Yeung, 2006). These forecasts call for further attention on how the trends of HFMD may evolve in the coming decades. In fact, warmer winters in recent years might help explaining the higher observed winter peaks of HFMD detected by the SSS in Hong Kong (Please see results found in Chapter 4). Our model in study (3) demonstrates that using climate parameters are associated with

HFMD activity. Based on the understanding that these meteorological parameters would affect activity of enteroviruses or host behaviour, this model can assist in better preparedness for the whole community before the actual upsurge of the disease.

The R_0 is an important parameter in understanding the epidemiology of many infectious diseases. The best illustration is SARS in 2003 when this new infection is introduced and became first known to human. It helps to predict how the epidemic would continue and assist in determining public health measures such as the duration of quarantine is sufficient or not. The present model in study (4) has estimated the R_0 of EV71 and CoxA16, which helps better understand the transmission dynamics of these important viruses in institutional or similar settings. Based on the results of study (4), one patient infected with EV71 would further infect 5.5 persons while those infected with CoxA16 would further infect 2.5 persons within the first incubation period. This is first ever reported in the published medical literature.

In investigating an outbreak, the number of patients affected in the subsequent incubation periods could be calculated from the formula mentioned in the method section. This would help the health authorities to decide appropriate control measures including school closure. Apart from severity of illness, the proportion of persons affected (attack rate) is one of the key factors in considering whether class suspension is necessary. If we adopt a similar approach to Singapore health authority, class suspension would be mandatory required to stop the transmission if there were certain number of HFMD cases or a pre-set attack rate is reached. Such criteria would also be very useful in risk communication and let the schools and parents get well prepared

for the necessary public health control measures.

In the last study, I demonstrated a reduction of transmission of HFMD during the SARS and H1N1 periods, which provides scientific evidence supporting that public health measures are effective in reducing the transmission of enteroviruses through respiratory droplets, faecal-oral, and direct contact from patients. Although these measures such as personal hygiene measures appeared to be simple and there are criticisms from the public that no specific measures were implemented during epidemic period, I was able to demonstrate and quantify their effectiveness. These results could be used in further risk communication and would increase the compliance of public hand washing, wearing facemask, not to bring their children to school if they were sick and other public health measures.

From study (5), I observed a larger and more sustained effect of public health measures on reduction of HFMD transmission during the SARS epidemic than the pandemic influenza H1N1 (2009). The lesson learnt here are the fright factors that would affect an individual's risk perception and behaviour. Fright factors are a number of emotional influences which increase public anxiety and influence public's response to the risk associated with a hazard. Novel source, poorly understood by science, involuntary exposure, arousing particular dread are examples of fright factors and are appropriate description for both viruses. Nonetheless, when more epidemiological data for pandemic influenza H1N1 (2009) were obtained by WHO and vaccine was available, public might perceive less threats and jeopardize the vigilance against the infection. Conveying appropriate risk communication is a vital

part of control measures in outbreak management, as stipulated by WHO (WHO, 2010). Further studies are needed to help us better understand how these psychosocial factors influence human behavior.

To conclude, by better understand the epidemiology of HFMD, I generate new knowledge which helps to formulate better prevention and control strategies for the disease. The following are recommendations are made based on the results of this thesis:

- The Health Authority should keep close monitoring the epidemiology of HFMD in Hong Kong, to assess if there is any further change in seasonal trends, susceptible population groups, clinical characteristics and complications, and laboratory findings on enteroviruses and molecular epidemiology of EV71 infection.
- To better prepare for the peak seasons of Hong Kong, the Health Authority should monitor meteorological parameters, apart from the traditional surveillance data on disease and laboratory.
- The Health Authority can make use of estimates the R_0 for EV71 and CoxA16, to predict how outbreaks of HFMD will spread and formulate control measures such as class suspension or school closure.
- In doing future risk communication, the Health Authority can explain to the public, that public health measures such as wearing face mask and hand hygiene are effective in reducing HFMD transmission. This can improve compliance of the public to these preventive measures.

The followings scientific studies are suggested to advance the knowledge for formulating better prevention and control strategies for HFMD:

- Social network analysis to study how HFMD is transmitted through interaction between infected patients including both children and adults.
- Effectiveness of specific public health measure, for example wearing face mask and proper hand hygiene, on reducing the transmission of HFMD in outbreaks
- Profiles of viral shedding in different routes of transmission (e.g. faeces, vesicles, nasal or oral secretions) to understand their relative importance in HFMD epidemics
- Disease incidence of HFMD including severity of the illnesses
- Role of environmental contamination and the effectiveness of cleansing and disinfection in halting outbreaks of HFMD

List of Publications Related to This Thesis

The results of this thesis have been published as original contributions in the following international peer-reviewed journals:

Ma E., Lam T., Chan K.C., Wong C., Chuang S.K. (2010) Changing Epidemiology of Hand, Foot, and Mouth Disease in Hong Kong, 2001 to 2009. *Japanese Journal of Infectious Disease 63*(6), 422-426.

Ma E., Chan K.C., Cheng P., Wong C., Chuang S.K. (2010). The enterovirus 71 epidemic in 2008--public health implications for Hong Kong. *International Journal of Infectious Disease 14*(9), e775-780.

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Ma E., Fung C., Yip S., Wong C., Chuang S.K., Tsang T. (2011) Estimation of the basic reproduction number of Enterovirus 71 and Coxsackievirus A16 in Hand, Foot, and Mouth Disease Outbreaks. *Paediatric Journal of Infectious Disease 30*(8), 675-679.

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