Risk, burden and control of non-polio enterovirus infections



Erhui Cai

St.Anne's College

University of Oxford

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Abstract

Non-polio enteroviruses are a large and diverse group of viruses that cause a wide spectrum of disease syndromes, ranging from mild, self-limiting cutaneous infections (hand-foot-mouth disease or herpangina), through to serious neurological complications such as encephalitis and life-threatening pulmonary haemorrhage or fulminant myocarditis. The international health risk posed by these viruses is demonstrated by the ongoing burden of enterovirus A71 in southeast and east Asia since its re-emergence in 1997, and the more recent re-emergence of enterovirus D68 in Europe and North America associated with severe respiratory disease and acute flaccid paralysis.

This thesis sets out to (i) improve preparedness for emerging non-polio enteroviruses by better characterising the public health risk they pose, and (ii) to improve the management of patients with moderate to severe disease by better describing the clinical epidemiology and by evaluating the performance of existing clinical case classification systems.

The thesis contains four results chapters. Chapter 2 presents a summary of newly emerged enteroviruses and their geographic distribution. This shows that whilst new enterovirus genotypes are regularly reported, the public health importance of these new genotypes appears limited, with greater morbidity and concern arising from the re-emergence of NPEVs that have been recognised for some time. This has implications for surveillance and pathogen characterisation activities. Chapter 3 reports the development, implementation and results of a novel tool to assess the public health risk of non-polio enteroviruses. The 'Enterovirus Risk Assessment Tool (ERAT) provides a robust and transparent ranking of the public health risk of non-polio enteroviruses and also provides new insights into critical research and knowledge gap. Chapter 4 reports the clinical features, disease progression, management and outcome of 6139 paediatric patients admitted to Zhengzhou Children's Hospital, China between Jan 1, 2015 and Dec 31, 2016. In particular, this chapter shows that although overall case fatality is very low (0.05%) many patients are classified as having complications and receive treatments, such as steroids, mannitol and intravenous immunoglobulin, whose value in these patients is as yet unproven. Chapter 5 identifies clinical case classification systems for hand-foot-mouth disease and explores their performance when applied to the clinical cohort

described in chapter 4. This chapter identifies significant problems with the existing case classification systems and makes recommendations for their improvement.

Overall, this thesis has generated and validated a new tool for prioritising non-polio enterovirus for research and development (the ERAT) and has generated new knowledge about the specific priorities for non-polio enterovirus research and development. The thesis also presents one of the largest and most complete analysis of patients hospitalised with hand, foot and mouth disease, identifying important questions about the optimal clinical management of these patients, which should be assessed in future clinical studies. Finally, the thesis provides the first critical appraisal of existing clinical case classification systems for hand, foot and mouth disease, identifying areas for improvement and further development.

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List of Abbreviations

ARI . . . . . Acute Respiratory Illness

AFM . . . . . Acute flaccid myelitis

AFP . . . . . Acute flaccid paralysis

AHC . . . . . Acute haemorrhagic conjunctivitis

CDC . . . . . Centre for Disease Prevention and Control

CEPI . . . . . Coalition for Epidemic Preparedness Innovations

CFR . . . . . Case Fatality Rate

CNS . . . . . Central Nervous System

CRF . . . . . Case Report Form

CSF . . . . . Cerebrospinal fluid

CV . . . . . Coxsackievirus

CV-A16 . . . . Coxsackievirus A16

CV-A24 . . . . Coxsackievirus A24

CV-A6 . . . . Coxsackievirus A6

ECDC . . . . . European Centre For Disease Prevention And Control

ELISA . . . . . Enzyme-Linked Immunosorbent Assays

ENPEN . . . . European Non-Polio Enterovirus Network

ERAT . . . . . Enterovirus Risk Assessment Tool

EVS . . . . . Enteroviruses

EV-A . . . . . Enterovirus A Species

EV-A71 . . . . Enterovirus A71

EV-B . . . . . Enterovirus B Species

EV-C . . . . . Enterovirus C Species

EV-D . . . . . Enterovirus D Species

EV-D68 . . . . Enterovirus D68

EV-D70 . . . . Enterovirus D70

EVD . . . . . Ebola Virus Disease

GAPDH . . . Glyceraldehyde 3-Phosphate Dehydrogenase

GBS . . . . . Guillian-Barré Syndrome

HFMD . . . . Hand-Foot-Mouth Disease

HA . . . . . Herpangina

ICTV . . . . . International Committee on Taxonomy of Viruses

IGA . . . . . Immunoglobulin A

IGG . . . . . Immunoglobulin G

IGM . . . . . Immunoglobulin M

IQR . . . . . Interquartile range

IRAT . . . . . Influenza Virologic Risk Assessment Tool

ICU . . . . . Intensive Care Unit

LRT . . . . . Likelihood Ratio Test

MCDA . . . . Multi-Criteria Decision Analysis

MCM . . . . . Medical Countermeasures

MESH . . . . . Medical Subject Headings

MOH . . . . . Ministry of Health

NCBI . . . . . National Centre For Biotechnology Information

NPEVS . . . . Non-Polio Enteroviruses

ORF . . . . . Open Reading Frame

PCIS . . . . . Paediatric Critical Illness Score

PCR . . . . . Polymerase Chain Reaction

PHE . . . . . Public Health England

PV . . . . . Poliovirus

RCR . . . . . Retrospective Chart Review

R&D . . . . . Research And Development

RNA . . . . . Ribonucleic Acid

RT-LAMP . . Reverse Transcription Loop-Mediated Isothermal Amplification

RT-PCR . . . Reverse Transcriptase Polymerase Chain Reaction

SARS . . . . . Severe Acute Respiratory Syndrome

TIPRA . . . . Tool for Influenza Pandemic Risk Assessment

UTR . . . . . Untranslated Region

VPG . . . . . Genome-like Virion Protein

WGS . . . . . Whole-Genome Sequencing

WHO . . . . . World Health Organisation

ZCH . . . . . Zhengzhou Children's Hospital

1

Introduction

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"The world is still ill-prepared
to respond to a severe influenza pandemic
or to any similarly global, sustained, and threatening
public health emergency."
– A new paradigm in global health security (2016) [1].

1.1 Non-polio enteroviruses

Enteroviruses are small non-enveloped RNA viruses in the genus of *Enterovirus* of the family *Picornaviridae* with a positive-sense, single-stranded genome. The members of the genus are abundant; more than 300 genetically distinct virus types are categorised into twelve *Enterovirus* species and three *Rhinovirus* species based on phylogenetic clustering [2, 3]. Of these, human viruses include members from *Enterovirus* A to D species and *Rhinovirus* A to C species; they are almost human exclusively pathogens inhabiting the alimentary and/or respiratory tract [4]. Human enteroviruses are separated into polioviruses, historically the most important enteroviruses, and non-polio enteroviruses. Despite the phylogenetic similarities between the two, only non-polio enteroviruses are discussed herein, as polioviruses are well-studied and subject to intensive eradication efforts [5].

In this thesis, the term 'enterovirus' (EV) is used to refer to human enteroviruses from *Enterovirus* A to D species for brevity unless otherwise specified. The term 'non-polio enterovirus' (NPEV) is used to refer to human enteroviruses other than polioviruses.

1.1.1 History

EV infections have been a scourge for humanity for centuries [4, 6]. Poliovirus (PV) is the prototypical EV for its ability to cause paralytic disease in children

and large outbreaks worldwide. Like other EVs, PVs cause a wide spectrum of clinical manifestations beyond the notorious poliomyelitis, however, due to the severe neurological sequelae, tremendous endeavour has been made to eliminate polio. Nowadays, polio is declining at a global scale, making NPEVs the most common viral cause of acute flaccid paralysis (AFP) [7]. NPEVs are also the most common cause of aseptic meningitis in children.

The discovery of the large and diverse group of EVs, to which polioviruses belong, came about following the investigation of polio outbreaks [6]. In 1948, members of the NPEVs, coxsackieviruses, were first identified from faeces of children with paralysis in Coxsackie, New York and named after the location of isolation. These isolates became the first prototype of group A coxsackie viruses, as they were antigenically distinct from polioviruses and produced myositis with flaccid paralysis in new-born mice but not in adult mice. In the following year, the first coxsackievirus group B was isolated in patients with aseptic meningitis. Clearly different from the group A coxsackieviruses in antigenicity, these isolates produced a distinct pathologic picture with spastic paralysis with involvement of brain, pancreas, heart and brown fat, which became the first prototype of group B coxsackieviruses. Later on, other genetically and antigenically distinct EVs were identified from stool in healthy volunteers and patients with a variety of disease and became the prototypes of echoviruses [6]. With the use of new-born mice and the development of tissue culture methods, there was a rapid growth in the number of identified EVs in the following decades. After the introduction of molecular detection methods, a more rapid expansion has been witnessed.

1.1.2 Virus structure and properties

EVs are small (27-30nm), non-enveloped, single-stranded positive-sense RNA viruses, enclosed in a capsid with icosahedral symmetry. The viral RNA genome is approximately 7,500 nucleotides in length, which is covalently linked to a genome-like

virion protein (VPg) binding at 5' end. The protein coding region is flanked by two untranslated regions (5' UTR and 3' UTR). The RNA encoded single polyprotein is nascently cleaved into three precursor molecules, namely P1, P2 and P3, which are subsequently processed into four structural (capsid proteins VP1 to VP4) and seven non-structural proteins (2A to 3D) during translation [4, 8].

One factor that distinguishes EVs from other picornaviruses is their stability in lower pH environments [9]. Being acid stable at pH values of less than 3.0 allows NPEVs to pass through the stomach and replicate in the intestine. EV infectivity is relatively resistant to many common laboratory disinfectants, such as 70% ethanol, isopropanol and dilute Lysol. Also they are insensitive to organic solvents due to the lack of a lipid envelope. EVs are relatively thermostable, although most of them are readily inactivated at 42°C. Some sulfhydryl reducing agents and magnesium cations can increase the stability at 50°C [4]. These physicochemical properties play an important role in the transmission of these viruses.

1.1.3 High-resolution structure of the virion

The viral capsid is composed of four structural proteins, with VP1, VP2, VP3 forming the shell and VP4 lying on the inner surface, according to the results of x-ray crystallography. VP1, VP2 and VP3 have the same topology but no sequence homology, whereas VP4 differs from them by having an extended conformation. VP1, VP2 and VP3 form an eight stranded, antiparallel β-barrel, which is a wedged-structure made up of two β-sheets. One β-sheet forms the wall of the wedge, and the other forms both the wall and the floor, with a bend in the centre. The interior of virions is formed by the N-termini of five VP3 making a cylindrically parallel β-sheet at the fivefold axis, surrounded by five three-stranded β-sheets formed by the N-termini of VP4 and VP1. Interaction between these two structures are mediated by the myristate group attached to the N-terminus of VP4.

Within the core of VP1, just beneath the canyon floor, is a hydrophobic pocket that maintains capsid dynamics and functions as binding site for antiviral drugs. On the surface of the virions, a prominent, star-shaped plateau known as mesa is found at the fivefold axis of symmetry, surrounded by a canyon and a protrusion at the threefold axis. It has been proved that the canyon acts as a receptor-binding site in a number of EVs [4]. While the connecting loops and the C-termini of the capsid proteins decorating the outer surface of the virion determine the viral serotypes.

To generate a virion, one copy of each structural protein (VP1-4) forms the basic building block named protomer. Protomer (5S) self assembles to pentamers (14S). Interaction among pentamers are stabilized by a seven-stranded β-sheet, composed of four β-strands of VP3 β-barrel and one strand from the N-terminus of VP1 that surround a two-stranded β-sheet made from the N-terminus of VP2 from a neighbouring pentamer. The 80S empty capsid that are assembled by 14S pentamers and the genomic RNA collectively form a 150S provirion. With the cleavage of VP0 molecules to VP2 and VP4, an infectious 160S virion is produced at the final step of morphogenesis.

1.1.4 Current classification

Based on antigenicity testing using neutralising antiserum and the pathogeneses in humans and in experimental animals, EVs were originally categorised serologically into four groups: polioviruses, coxsackie A viruses, coxsackie B viruses and echoviruses [10, 11]. However, as the number of recognised virus serotypes grew, the antigenic groupings became increasingly complicated and sometimes failed to detect the serotype of an isolate because of antigenic shift, recombination, or the presence of virus mixtures in the specimen.

More recently, EVs were grouped into four genetically different species, designated as *Enterovirus* A (EV-A), *Enterovirus* B (EV-B), *Enterovirus* C (EV-C) and

Enterovirus D (EV-D) [12]. EVs were further differentiated into (sero)types by the degree of sequence divergence of structural gene regions such as VP1, which contains the major neutralising epitopes and correlates well with serotypes [8, 13, 14]. Currently, more than 110 genetic EV types (around 75 serotypes) have been identified [2]. The Enterovirus A species consists of the most important EV types associated with large epidemics of hand-foot-mouth disease (HFMD). Table 1.1 shows the members by *Enterovirus* species.

Table 1.1: Human enterovirus (sero)types by species. Traditional and current taxonomy for human enterovirus genus suggested by the International Committee on the Taxonomy of Viruses.

Traditional classification		
Species	No.	serotype [1]
Poliovirus	3	PV(1-3)
Group A coxsackievirus	23	CV-A(1-22,24)
Group B coxsackievirus	6	CV-B(1-6)
Echovirus	28	E(1-7, 9, 11-21, 24-27, 29-33)
Enterovirus	4	EV(68-71)
Current classification		
Species [2]	No.	(sero)type [3]
Enterovirus A	21	CV-A(2-8, 10, 12, 14, 16) EV-A(71, 76, 89-92, 114, 119, 120, 121)
Enterovirus B	62	CV-A9 CV-B(1-6) E(1-7, 9, 11-21, 24-27, 29-33) EV-B(69, 73-75,77-88, 93, 97, 98, 100, 101, 106, 107, 110-113)
Enterovirus C	23	PV(1-3) CV-A(1, 11, 13, 17, 19-22, 24) EV-C(95, 96, 99, 102, 104, 105, 109, 113, 116-118)
Enterovirus D	5	EV-D(68, 70, 94, 111, 120)

CV: coxsackievirus, EV: enterovirus, E:echovirus, PV: poliovirus.
[1] Currently both human and non-human EVs are classified into 13 species. Of these, more than 110 EVs that cause human infections have been identified and designated as *Enterovirus A-D* spiecies [3, 15, 16]. Some EV (sero)types have been reclassified. Specifically, E-10, E-22, E-23, E-28 and EV-72 have been reclassified into other genera. Human rhinovirus 87 has been found to be a strain of EV-D68. CV-A23 is the same serotype as E-9. Similarly, E-8 is the same serotype as E-1, and CV-A15 is the same serotype as CV-A11, and CV-A18 is the same as serotype as CV-A13 [2].
[2] Polioviruses have been merged into *Enterovirus* C species.
[3] VP1 nucleotide sequence identity of at least 75% (85% amino acid identity) between an isolate and a EV prototype strain suggests evidence of serotypical similarity to the prototype [8]. Some EV serotypes have not been recognised by the International Committee on Taxonomy of Viruses.

1.1.5 Laboratory diagnosis

A range of laboratory diagnostic methods have been developed to support case identification and management and disease control. The choice of diagnostic method depends on the purpose for which the testing is done (i.e., clinical diagnosis, epidemiological survey, or vaccine development).

For the detection of acute NPEV disease from clinical samples, reverse transcriptase polymerase chain reaction (RT-PCR) targeting the 5' UTR is utilised because of its high sensitivity, high specificity and rapid turn-around time [14, 17]. Two kinds of RT-PCR assays have so far been used for clinical specimens. The pan-enterovirus assays have broad specificities. They employed primers or probes matching highly conserved sequences in the 5' noncoding region to recognise nearly all EVs and antigenic variant strains and the *GAPDH* gene of host cells as an internal control [14, 18, 19]. These primers have broader targeting specificities; they can be used to screen the presence of NPEVs in samples. Monospecific RT-PCR assays were used for genotype differentiation of circulating NPEV. Primers or probes targeting different portions of VP1 enabled the identification of the most prominent NPEV, because of the excellent correlation between the VP1 sequence and the enterovirus serotypes. The diagnostic performance of these generic and genotype-specific RT-PCR tests usually have a sensitivity of 90-95% and a specificity of 90-100%, with a detection limit of 10 to 10^3 copies/ml [18, 19].

Traditional virus isolation is performed using time-consuming cell culture techniques. The virus neutralisation test is based on the typical cytopathic effect of certain NPEVs on cell culture cells and performed using internationally standardised hyper-immune antisera. The virus neutralisation test is also used to measure the prevalence and titres of neutralising antibodies in the population [20, 21]. In addition, enzyme-linked immunosorbent assays (ELISAs) for NPEV-specific IgM have been developed for epidemiological studies. Whilst these IgM-ELISAs are

sensitive and specific for enterovirus infections, they are not strictly serotype specific, and will detect heterotypic antibodies from other NPEV infections. The identification of NPEVs (i.e. new EV types or new recombinant forms of known EVs) that cannot be typed are restricted to reference laboratories that perform further genome sequencing [13, 14].

Clinical specimens for isolation of virus or RT-PCR-detection are stool samples, rectal swabs, throat swabs, vesicle swabs, vesicle fluid, blood and cerebrospinal fluid (CSF). In some studies, stool had a higher diagnostic yield (89%) than rectal and throat swabs (77% and 74%, respectively) [22, 23]. Blood and CSF have the added advantage of being sterile sites, but they are subject to low diagnostic yield [14].

1.1.6 Transmission

NPEVs are transmitted from person to person predominantly by the faecal-oral route and less frequently by the respiratory route through nasal secretions, saliva or sputum [4]. Transmission modes may vary in different regions, with faecal-oral route predominating in areas where sanitary conditions are poor and respiratory transmission in more developed countries [4]. Other modes of direct person-to-person transmission, via vesicle fluid and the eye mucosa, have been reported [24].

The incubation period is typically around 3-5 days [9]. Primary infection with an EV leads to viral replication in the upper respiratory tract or gastrointestinal tract, followed by a transient viremia and sometimes migration into other tissues [4]. Extra-gastrointestinal NPEV infection is dependent on host factors such as immunosuppression, but also on the presence and distribution of specific receptors for the particular viral capsid proteins [25].

Transmission most often occurs within communities and in day-care centres and nurseries. Human NPEVs have been isolated from different environmental

sources (surface and underground water) [4].

1.2 General epidemiology

NPEV infections are very common and have a worldwide distribution. Global communities with NPEV infections report a multidimensional burden, as these viruses can result in a wide array of illnesses, some easily recognised clinically and other diagnosed as non-specific viral syndromes. Young children and infants are disproportionately affected and associated with more severe syndromes, such as sepsis-like disease, aseptic meningitis, encephalitis, acute flaccid paralysis and myocarditis [26, 27].

The global disease burden of NPEV infections has not been fully characterised, in part due to the lack of global surveillance and monitoring systems. In the US, it is estimated that 10 to 15 million symptomatic NPEV infections occur per year [28]. In China alone, approximately 1 to 3 million infections per year were reported for hand, foot and mouth disease (HFMD), the most common clinical syndrome associated with NPEV in Asia [29]. Beyond Asia, the disease burden remains largely unknown, because infections are either subclinical or frequently occur as part of outbreaks with no systematic surveillance system in place [30].

Generally, the epidemiological features of NPEV infections vary by time, geographical region, age group and clinical syndromes on a global scale. From studies conducted in health-care settings, the annual incidence was estimated at 26 to per 100,000 neonates under the age of 30 days to 120 per 100,000 children under the age of 18, despite geographical variations and constellations of syndromes [31, 32].

Evidence on epidemiological studies based on serological or molecular methods also suggested a large proportion of underreported NPEV infections. Such data about NPEV infections is largely derived from hospitalised cases, the reported

epidemiological features are based on multiple studies with different methodologies and populations [26]. A seroepidemiological study in the Netherlands found that 9.5% of a total of 1273 enterovirus isolates were detected from infants under 30 days of age, of whom 35% had a clinical diagnosis of sepsis, 24% of meningitis and 21% of fever without source. As the study sub-sites tended to enrol patients with severe forms of diseases, a risk of underreporting mild diseases could not be ruled out [31]. A more recent cohort study using molecular detection method reported a higher proportion of neonates with fever without source than sepsis and meningitis (51% vs 11% and 38%) [27].

The overall case-fatality rate of NPEV infections is usually low in the general population, but it could vary slightly at different transmission stages and in different syndromic surveillances. For HFMD associated with any NPEVs, case-fatality rate was estimated to be around 1.2% during the infancy of the HFMD epidemics in 2008 and reduced to 0.03% and 3.0%, respectively, according to national surveillance data in China [32, 33]. In comparison, case-fatality rates were reported to be approximately 3% for more severe syndromes associated with NPEVs [26]. Higher case-fatality rates were reported in specific age groups. The highest case-fatality rate of HFMD was recorded in infants aged 6-11 months who had an EV-A71 infection (97 per 100,000 infants), and declined in older children [32, 34].

Each year, EV infections present with a clear seasonal pattern with the highest incidence is in the summer and autumn. The summer-fall seasonality is characterised by a single peak in northern latitudes and two semi-annual peaks at lower latitudes [32, 35]. Transmission may occur all year round in tropical regions, with a proportion of 78% to 83% NPEV infections detected during the peak months, usually from May to December [26, 31].

In terms of long-term trends of circulation, two major patterns exist: epidemic or endemic. NPEVs with an epidemic pattern of circulation were characterised by substantial fluctuations in circulation levels over time, with a cyclical pattern of

2-5 years and large peaks when the NPEV was the more prevalent type for a given year [23, 26]. Whereas NPEVs with an endemic pattern had stable and usually low-levels of circulation with few distinct peaks [26].

Transmission and the distinctive epidemiological pattern of NPEV infections are determined by the inherent transmissibility of the pathogen (measured by the basic reproductive number R_0) [36]. R_0 is an epidemiologic metric to describe the transmissibility of an infectious agent. It is a population-specific indicator, which may be affected by the duration of contagiousness following infection, the likelihood of infection per contact between a susceptible person and an infectious person, and the contact rate [37]. Such variations have been seen in different countries, where R_0 for EV-A71 and CV-A16 was estimated to be 26.6 and 27.2, respectively, in a modelling study using the surveillance data in China [38]. R_0 for EV-A71, CV-A16 and CV-A6 in Singapore and Hongkong was estimated to be 3.5, 2.4-2.5, and 5.0-5.5, respectively [39, 40]. When the R_0 has a value of more than one, an outbreak or epidemic is expected to continue to grow. The magnitude of R_0 also reflected the potential size of the outbreak or endemic. To slow the spread of infection by vaccination or natural infection, the herd immunity threshold must be achieved. The herd immunity threshold is calculated by $1\text{-}1/R_0$, therefore, assuming an R_0 of 27, the corresponding immunity threshold for prevention an epidemic should be greater than 90% [41].

The transmission of NPEV infection may also be driven by the number of infections occurring prior to the onset of overt clinical symptoms (or via asymptomatic infections), the proportion of susceptible populations, and the proportion of the recovered [36, 42]. In a family cohort study, 183 children were infected with EV-A71 among 94 families (433 family members), 6% of whom were asymptomatic. In contrast, 87 adults were infected, 53% of whom were found asymptomatic. This indicated that asymptomatic adults were be a potential source of transmission [14, 43]. One key factor that contributes to the transmission and multi-year cycles is

the introduction of a new susceptible population in new births cohorts [5]. Another factor that shape the transmission dynamic is the intraserotypic and interserotypic immunity reaction. Although the actual degree of cross-reaction associated with a primary EV infection is uncertain. There is clear evidence for incomplete protection against another EV serotype as sequential (secondary) infections with different serotypes have been observed [42, 44].

1.2.1 Clinical presentation and syndromes

NPEVs have been implicated in a wide spectrum of clinical disorders involving multiple organ systems, ranging from mild to severe presentations. NPEVs are the causal agent of herpangina, hand-foot-mouth-disease (HFMD); myositis, pleurodynia, acute inflammatory muscle disease; upper respiratory infection, pneumonia; acute haemorrhagic conjunctivitis; acute and chronic cardiac disease (myocarditis and pericarditis); aseptic meningitis, AFP, Guillain-Barré syndrome (GBS), encephalitis and neonatal enteroviral sepsis. Noticeably, the same clinical syndromes can be caused by a range of different NPEVs.

Often illnesses are nonspecific (e.g. acute respiratory illnesses), and the EV aetiology remains undiagnosed. NPEVs have been also implicated in causing chronic diseases, such as sporadic human dilated cardiomyopathy and type 1 diabetes mellitus [45].

HFMD presents with fever and sore throat. Vesicles typically appear after one or two days on the mucuous membranes of the mouth and tongue, as well as on palms of the hands and soles of the feet. Further vesicles may occur on the buttocks, elbows and knees. The skin lesions are tender and show a surrounding erythema. HFMD is usually mild and self-limiting, however, severe complications in young children such as meningitis, AFP, brainstem encephalitis and death have been

reported. EV-A71 infection has been associated with severe illness [46]. HFMD has become an important public health problem in the Asia-Pacific region.

1.2.2 Notable outbreaks of NPEV infections

As the first documented NPEV pandemic, enterovirus D70 (EV-D70) caused acute haemorrhagic conjunctivitis (AHC) in 1969 in Ghana, after which the virus spread globally, affecting millions of people [47]. Another main causative agent that leads to AHC is coxsackievirus A24 (CV-A24), with two pandemics occurring in 1985 and 2002 respectively [30].

Recently, notable outbreaks and epidemics have been caused by EV-A71 and enterovirus D68 (EV-D68). Outbreaks of EV-71 were initially reported in the USA in 1969. Historically, outbreaks of EV-A71 have been sporadic and local [33, 48]. A changing pattern of worldwide epidemiology began in 1997, when unprecedented numbers of EV-A71 associated HFMD cases with initially high case-fatality rate (CFR) occurred for the first time in Malaysia and in Taiwan [33]. Since then, HFMD epidemics caused by EV-A71 have expanded significantly with a high number of cases. In China, more than 10 million cases of HFMD and more than 3000 deaths were reported from 2008 to 2014, with 80% of severe cases and 93% of fatal cases attributable to EV-A71 [49]. Outside the Asia-Pacific region, EV-A71 has continued to circulate at a low level in Africa, Europe and the USA.

First reported in 1962, EV-D68 was first isolated from throat swabs among children hospitalised with pneumonia and bronchitis in Berkeley, California [50]. Sporadic cases with few fatalities have been reported until recently. An increase in respiratory disease associated with EV-D68 has been reported in a series of outbreaks in North America, Europe and Asia since 2009 [51]. In the summer and fall of 2014, 2,287 cases of EV-D68 were reported in 20 countries, associated with severe respiratory disease. Concurrently, clusters of children with acute flaccid

paralysis of unknown cause were reported [52]. In an outbreak of respiratory illness reported in Sweden in 2016, ten patients had respiratory failure or bulbar symptoms and one case was fatal [53]. Increased detection of EV-D68 have been reported in the Netherlands and Germany [54, 55]. Notably, Denmark, France, the Netherlands, Spain, and United Kingdom (Wales) have reported severe infections associated with a variety of different strains of EV-D68 in 2016 [53, 54, 56].

1.3 Prevention, treatment and control

1.3.1 Immunity

The humoral immune response plays an important role in the elimination of NPEV infection. Innate immune responses are activated through pathogen recognition receptors (i.e. Toll-like receptors or retinoic acid-inducible gene-like receptors) and help to start nonspecific defense mechanism immediately or within hours of an EV antigen's appearance in the body [57, 58]. Enterovirus infection then induces a rapid production of heterotypic immunoglobulin M (IgM) class antibodies turning to class immunoglobulin G (IgG) and immunoglobulin A (IgA) response. Specific neutralising antibodies are formed a few days after onset of disease. IgG and IgA class antibodies may persist for years, while IgM responses usually only last a few months [59]. Re-infection with EVs provokes a secondary response with rises in specific IgG titres accompanied with titres rise of other related enterovirus antibodies. In young children protected by maternal antibodies, seropositivity decreased gradually to a nadir of 10% at 1 year of age, before climbing back again to 92% in 11- to 15-year-old children [20, 60].

Previous studies have suggested that humoral immune responses are different after NPEV primary infections and vaccination. A study evaluating EV-A71 and CV-A16 specific IgM from serum samples of patients with primary infections detected

cross-reactivity in EV-A71 and CV-A16 IgM ELISAs. EV-A71 IgM was positive in 31% CV-A16 infections and in 29% other EV infections. Similarly, CV-A16 IgM was positive in 28% EV-A71 infections [61]. Laboratory evidence also supported that weak cross-reactivity against other NPEV serotypes might exist. In comparison, cross-protection from vaccination seemed limited due to the high variability of the viral proteins between EV-A71 and vaccine strains [62, 63].

Other studies showed that cross-neutralising antibody response could be elicited against several EV-A71 subgenotypes, namely B1, B4, B5, C2 and C4a and C4b in individuals vaccinated with an inactivated B4 subgenotype [62]. Moreover, C4 genotype EV-A71 vaccines in clinical trials elicited broad cross-neutralising antibodies against EV-A71 subgenotypes C2, C4, C5, B4 and B5. But these inactivated EV-A71 vaccine could not provide cross-protection against other common NPEV subtypes associated with HFMD. Similarly, recent case control studies evaluating the effectiveness of EV-A71 vaccine among medically attended children with HFMD showed that no statistically significant difference was observed in the occurrence of other NPEV infections (such as CV-A16, CV-A6, other non-EV-A71, non CV-A16 and non CV-A6 NPEVs) between the vaccinated and non-vaccinated group [63, 64].

1.3.2 Non-pharmaceutical interventions

Primary prevention of NPEV infections is currently possible with vaccination only for EV-A71. In China, phase III trials of an inactivated vaccine against EV-A71 C4 subgenotype have shown vaccine efficacy of 97%, with an acceptable safety profile [65]. This vaccine has been licensed since 2015 [65, 66]. Recent data show the vaccine has an effectiveness of 91% against non-severe disease and 73% against severe disease in children aged less than 6-year-old. Although other studies suggested cross-protection against other EV-A71 subgenotypes (B4, B5, C2 and C5), there is no evidence for cross-protection against CV-A16 and coxsackieviru A16 (CV-A16) [63, 67].

As of 2019, the EV-A71 vaccine is still a private sector vaccine in China. Multiple provinces have started to provide the EV-A71 vaccine since 2017. However, there is no direct estimate of the uptake of the vaccine and limited evidence on vaccine efficacy. It was noticed that the actual uptake of the vaccine remained low in individual provinces, ranging from 10% in provinces with low incidence rates of HFMD to 50% in provinces with high incidence rates. An increase in the vaccine uptake coincided with a decrease in the number of symptomatic and severe cases in 2017 [68].

Aside from vaccination, public health measures can reduce the transmission of viruses and hand hygiene is an effective way to reduce the spread of both respiratory and enteric infections [69]. A case control study investigating the effectiveness of hand washing in outbreaks indicated a more than 95% decreased risk of developing HFMD or herpangina, after adjusting for residency, age and community exposures [70].

Two other simple public health measures can be used to control outbreaks, namely isolating symptomatic individuals and tracing and quarantining their contacts [36]. Because these measures depend on the recognition of specific disease syndromes, the success of controlling outbreaks is dependent on the timing of infectiousness and the onset of symptoms [36]. In reality, there is a time lag between a patient becoming ill and being isolated. The delay will reduce the efficacy of the control measure. The effectiveness of such self-isolation depends on the specificity of symptoms and their timing in relation to infectiousness [36]. In an outbreak setting, prodromal fever, skin rashes or respiratory symptoms could be used to screen patients and initiate control measures.

When community transmission or an institutional outbreak occurs, schools can be temporarily closed. A modelling study in Singapore suggested that this social distancing intervention reduce the number of new cases by 1204 from 2011

to 2016, an average 2% reduction compared with the total number of cases during that period [71].

1.3.3 Pharmaceutical interventions

Treatment of NPEV remains largely supportive, focusing on the early detection and management of the most severe complications. Intravenous immunoglobulin (IVIG) has been used in immunocompromised individuals and patients with severe symptoms, without good quality evidence to substantiate its use [72, 73]. There are no specific antiviral drugs for NPEV infection; however, ribavirin has been widely used in China.

Pleconaril is a novel therapeutic option that is most promising and advanced in clinical testings. Pleconaril is an oral viral capsid inhibitor with metabolic stabilisation, binds with high affinity and specificity to a hydrophobic pocket in the viral capsid inducing conformational change that lead to altered receptor binding and viral coating [74]. Pleconaril is orally bioavailable and achieves serum concentrations in excess of those required to inhibit 90% of clinical rhino- and enteroviral isolates in vitro. It possesses the additional advantage of achieving several fold higher concentrations within the central nervous system and nasal secretions than in serum, a characteristic that is highly desirable for an antiviral targeted towards viruses known to cause central nervous system and upper respiratory tract infections. Approximately 80% of an orally administered dose is excreted in the faeces within 48 hours. Urine excretion accounts for the remainder of the drug [74].

Pleconaril was generally well-tolerated in patients with NPEV-associated meningitis, upper respiratory tract infections, sepsis and other severe diseases [75]. Pleconaril has demonstrated an excellent safety profile in dose escalation and clinical studies [74, 76]. A small compassionate use study found that an oral pleconaril treatment (5.0mg/kg 3 times per day for children, or 200mg/400mg

3 times per day for adults for 7 to 10 days, with or without a second course of therapy) had beneficial effects in patients with potentially life-threatening EV infection (such as EV meningoencephalitis, neonatal EV sepsis, and myocarditis, etc). Favourable clinical, virological, laboratory, and radiological responses were observed in 78%, 92%, 88% and 60% of patients, respectively. Around 60% of the participants reported 56 adverse events in total [77]. In two randomised controlled trials of 1363 patients with picornavirus-associated common cold, an association was found between the oral pleconaril treatment (400 mg 3 times per day for 5 days) and reduced time to alleviation of illness, lower cold symptom severity score and frequency of pathogen cultured from nasal mucus specimens at day 2. The most commonly reported adverse events were headache, diarrhoea and nausea. More than 95% of the adverse events were mild or moderate in severity [78]. A phase 2 clinical trial using pleconaril (5.0mg/kg per dose of liquid formulation), showed no difference in day 5 oropharyngeal culture positivity in neonates with suspected EV sepsis. But it was found that the use of pleconaril was associated reduced time to culture negative from all anatomic sites combined and fewer PCR positive swabs from oropharynx compared with the placebo arm [75].

1.4 Problem statement

Recent outbreaks have highlighted the public health impact of NPEVs [12]. From the late 1990s onwards, Southeast Asia has become as an epicentre for HFMD due to its notably high epidemic activity. The emergence of the B3-B5 lineage of EV-A71 in Southeast Asia in 1997 and its association with severe disease, followed by the emergence of the C4 subgenotype is well recognised. The emergence of EV-A71 C4 lineages, the most neurotropic NPEV except poliovirus, was detected following the investigation of a cluster of deaths from rapid progressive pulmonary oedema/haemorrhage in unprecedented numbers of children with HFMD in Malaysia

and Taiwan, respectively [33, 79–81] . A series of similar outbreaks and epidemics with thousands of cases were found elsewhere in Southeast Asia thereafter [33, 82].

Prior to 2004 HFMD was largely limited to southern China, with EV-A71 subgenotype C4b dominating since 1998 [83–86]. From 2004 onwards, epidemic activities and endemic circulation increased in southern China, followed by a rapid spread of epidemics across central and northern China. This coincided with the replacement of a previously circulating EV-A71 subgenotype C4b with C4a, the latter became predominant in 2007 [83–86]. Since then, a clear pattern, coupled with rapid increases in incident cases and epidemics with more severe forms of HFMD, continued to take shape. As of 31 December 2017, a total of 17,908,343 cases, with at least 3,526 fatalities were officially reported in China [29, 87].

Towards the end of 2014, Enterovirus D68 (EV-D68) gained the attention of the global community as well as international health authorities. An upsurge of hospitalisations and admissions to intensive-care units (ICU) with severe respiratory illness was reported, particularly among children with asthma. Shortly afterwards, the outbreak spread to Canada, Europe and Asia. Concurrently, clusters of children with acute flaccid paralysis of unknown cause were also reported in the United States and Europe. Concerns about a potentially uncontrollable epidemic of severe lower respiratory illness and neurological complications associated with EV-D68 were prompted [52, 88].

The very large diversity of enterovirus types, their high genetic diversity through genetic mutation or recombination events, their high transmissibility, and their proven ability to cause severe disease makes this groups of viruses a global health threat. In addition, the lack of effective vaccines (other than the EV-A71 C4 vaccine) and specific treatments adds to the concern about NPEVs.

In response to the 2014 west Africa Ebola crisis and the lack of effective diagnostics, treatments and vaccines, the World Health Organisation (WHO)

developed the Research and Development Blueprint for action to Prevent Epidemics (the Blueprint). One component of the Blueprint is the generation of a list of high-threat diseases that lack effective countermeasures: these are termed Blueprint Priority Diseases [89]. In the second annual review of Blueprint Priority Diseases in 2018, emergent non-polio enteroviruses (including EV-A71, D68) were considered for inclusion. Although not making the main list the WHO state that *"These diseases pose major public health risks and further research and development is needed, including surveillance and diagnostics. They should be watched carefully and considered again at the next annual review. Efforts in the interim to understand and mitigate them are encouraged."* [90].

1.5 Aim and structure

The overarching aims of the work presented in this thesis are to:

1. to perform a structured analysis to identify newly emerged NPEVs and visualise their geographic distribution.

2. to develop a transparent, systematic and replicable algorithm to assess the public health risk of NPEVs.

3. to describe the epidemiology, clinicopathological features, disease progression, case management and outcome in children hospitalised with HFMD.

4. to evaluate the usefulness of the existing HFMD clinical case classification systems and make recommendations on their use and further development.

1.5.1 Thesis structure

Chapter 2 reports the findings of a review of newly-identified NPEVs and their geographic distribution.

Chapter 3 reports the results of research to develop, pilot and implement a non-polio virus risk assessment tool, which aimed to characterise the relative public health risk of a range of NPEVs and to identify gaps in research and development preparedness.

Chapter 4 reports the conduct and results of a large retrospective cohort study of consecutive paediatric patients hospitalised with a clinical diagnosis of HFDM in Zhengzhou Children's Hospital in China in 2015 and 2016. The epidemiology, clinical features, disease progression, management and outcome of over 6000 patients are described. Both univariate and multivariable statistical analyses are performed to identify factors associated with severe disease.

Chapter 5 describes efforts to evaluate different national and international clinical case classification systems for HFMD. Major clinical case classification systems are identified through a literature search and then applied to the retrospective cohort dataset described in the chapter 4. The ability of the clinical case classification systems to correctly identify patients requiring intensive medical management is assessed.

Impact of the work

Sections of this chapter have been adapted and were submitted as a summary to the WHO 2018 Second annual review of the Blueprint list of priority diseases. As a result, emergent non-polio enteroviruses were placed on the "watch-list".

2

Diversity and distribution of newly emerged NPEVs

Contents

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2.1 Preface

The objective of this thesis is to provide a structured summary of newly emerged NPEVs and re-emerged NPEVs.

2.2 Author's contribution

For the work in this chapter I conceived the literature review, undertook the study selection of all citations for inclusion, extracted the data, and compiled the database for analysis. I undertook all data analysis, produced all tables and figures, and wrote the text in this chapter.

2.3 Abstract

The characteristics and epidemiology of newly emerged and re-emerged NPEVs have not been well studied. To summarise the characteristics and epidemiology of the newly reported NPEVs in humans and non-human primates infections. A literature search was performed using PubMed. Additional newly reported NPEVs were sought from the GIDEON database and the International Committee on Taxonomy of Viruses (ICTV) website. Independent extraction of articles and data was performed by a single investigator using predefined data fields. The location of each included isolates was geocoded and visualised using ArcGIS. The PubMed literature search NPEVs yielded 1179 articles. In total, 67 articles and grey literature were included in the full text literature review. Of these, 38 articles reported 112 isolates that represented 44 newly-identified NPEV sero/genotype in humans and non-human primates. New NPEV genotypes are regularly reported, however the public health importance of these new genotypes appears limited, with greater morbidity and concern arising from the re-emergence of NPEV that have been recognised for

some time. This suggests that improving preparedness for NPEVs should focus on existing NPEV with changing epidemiology, rather than the detection of new genotypes. The mechanisms by which known NPEVs cause an increase in morbidity should be the focus of further research.

2.4 Background

NPEVs have been recognised as important agents of disease since their discovery in the 1950s. Initially, NPEVs were characterised and classified based upon their antigenic similarity as assessed by neutralisation by antiserum and their pathogenic properties (See section 1.1). Since the late 1990s, the use of molecular diagnostic methods has changed the way in which NPEVs are typed and classified [11, 91, 92]. The International Committee on Taxonomy of Viruses (ICTV) defines members of a species of the genus *Enterovirus* as having: (i) a significant degree of amino acid identity of the P1, 2C, 3C and 3D proteins; (ii) monophyly in phylogenetic trees; and (iii) essentially identical genome maps. NPEVs are now largely classified based on the sequence of the genome region that codes the VP1 protein, which is the main target for neutralising antibodies. This method correlates well with serological methods and allows classification of viruses that are not easily cultured. Divergence in the VP1 nucleotide sequence of >25% correlates with serologically distinct types [91]. NPEVS are now classified based on VP1 genotyping without reference to serology and the term serotypes is increasingly being replaced by the term 'genotype' or 'type'.

The introduction of molecular methods has led to an expansion from round 66 serotypes > 300 genotypes [91]. However, the extent to which the identification of new genotypes reflects any material change in the risk to human health is unclear. To our knowledge there has been no systematic review of newly identified NPEVs and their implications for human health.

2.5 Aim and objectives

The aim of this work is to provide a structured summary of the characteristics and epidemiology of newly reported NPEVs.

2.6 Materials and methods

Literature search

PubMed was searched from January 01, 2005 to December 31, 2015 using the following term: novel[All Fields] AND ((“enterovirus”[MeSH Terms] OR “enterovirus”[All Fields]) OR (“enterovirus”[MeSH Terms] OR “enterovirus”[All Fields] OR “coxsackievirus”[All Fields]) OR (“enterovirus b, human”[MeSH Terms] OR “human enterovirus b”[All Fields] OR “echovirus”[All Fields])) AND (“2005/01/01”[PDAT] : “2015/12/31”[PDAT]). The starting year of 2015 was chosen since it roughly corresponds with the more widespread application of the molecular diagnostic method based on the correlation of serotype and NPEV VP1 sequence [91–96]. The end year of 2015 was chosen as the review was conducted in 2016 and this provides a full ten years of data. There was no limitation on study type. Only Chinese and English articles were included.

In addition, the PubMed search was supplemented by: (i) screening of postings on enteroviruses in the GIDEON database over the same period; (ii) searching the website picornaviridae.com for new genotypes with a sequence reference first uploaded over the same period.

Eligibility criteria

Published studies identified through PubMed were included if they met any of the following criteria: (1) reported the identification of enterovirus isolates or strains that were antigenically distinct from any other previously known NPEV; (2) identification was based on the use of nucleic acid detection, virus culture, serology, or any other established diagnostic test [97]. (3) NPEV detection in humans and non-human primates were included but detections in other animal species were excluded.

The studies were excluded if they met any of the following criteria: (1) the study was not published in the specified time frame; (2) the study reported poliovirus only; (3) the strain is previously known as a predominant serotype or its genotype or subgenotype for epidemics or endemics, such as EV-A71, CV-A16 or EV-D68; (4) the strain or isolate was not genetically, spatially or temporally representative (i.e., the use of any pre-existing reference or commercial strains, or any transformed strains); (5) taxonomic classification was only available in the level of species or genus; (6) the strain was previously reported elsewhere; (7) the study was secondary research and did not provide nucleotide sequences on the isolation itself.

The potentially relevant isolates or strains were further excluded if they met any of the following criteria: (1) they were tentative members previously listed in the targeted genus, but had been reclassified into other genera; (2) they were considered identical to an existing serotype. Time frame was selected because proposals for taxonomy were not accepted until a final 'ratification' was voted by the full ICTV membership [97]. No limitation was imposed on specimen type.

Data extraction

Study selection was performed by one reviewer based on the pre-specified eligibility criteria described above. A standardised data extraction sheet was develop to

collect the information as follows: the study ID, first author, year of publication, strain ID, the location of identification, genus and serotype, type of host, infection status (e.g. an AFP surveillance sample, gastroenteritis, pneumonia), and type of specimen (e.g. stool, nasopharyngeal swab).

Geocoding and mapping

The location of a reported isolate was converted into geographic coordinates using online 'batch geocoder' [98]. As sparse data was expected, geographic information of individual strains was aggregated at national level using choropleth maps [99]. Microsoft Excel 2016 was used to manage data and ArcGIS 10.5 (ESRI, Redlands, CA) was used to visualise the geographical distribution of the targeted NPEVs.

2.7 Results

Literature search

The literature search yielded a total of 1179 articles, with 744 articles from PubMed and 435 reports from GIDEON and ICTV. Sixty seven studies were included in the final data extraction. Notably, the GIDEON database whilst providing outbreak information, did not provide any useful genotype or strain data. The flow chart of the study and isolate selection is presented in figure 2.1.

Newly reported isolates and genotypes

One hundred and twelve separate isolates representing 44 different sero/genotypes of enterovirus were reported.

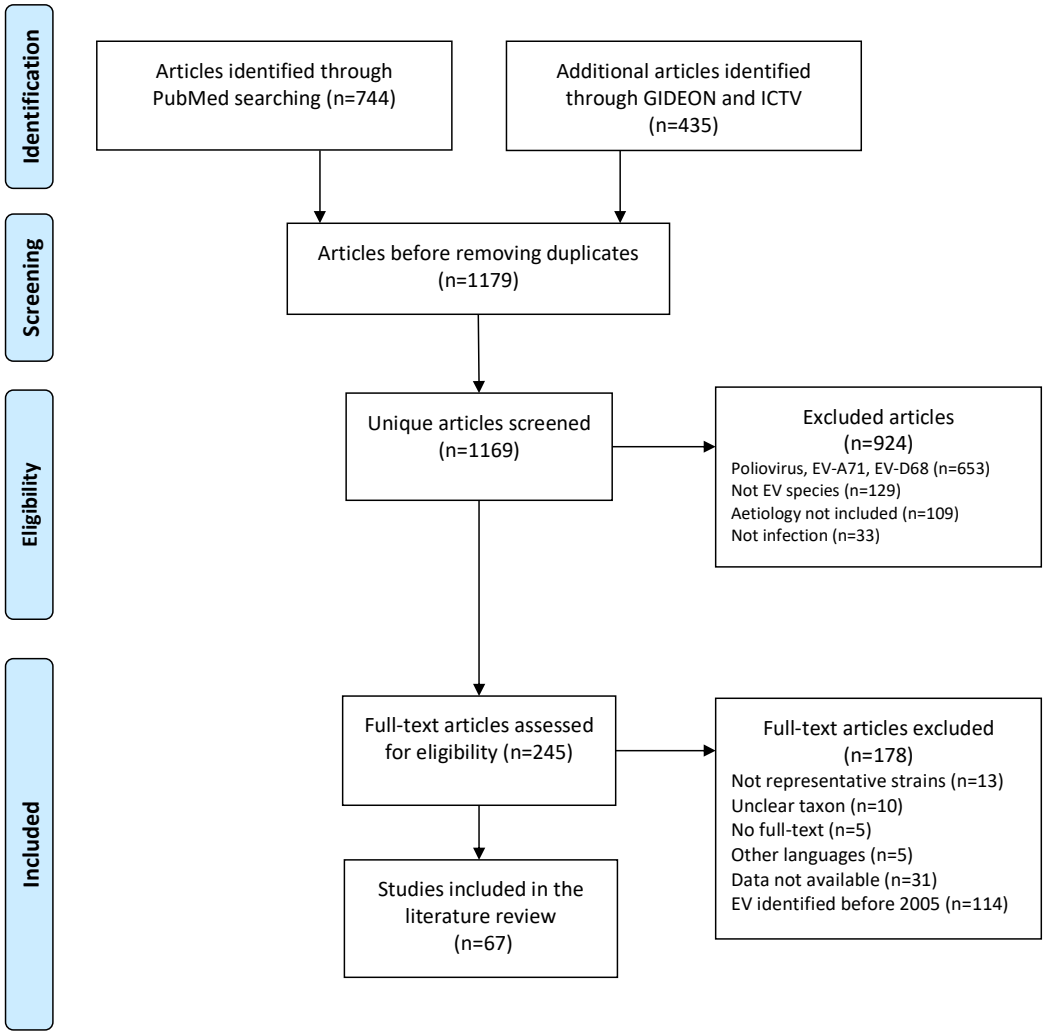


Figure 2.1: Flow diagram describing the study screening and inclusion for newly-emerged NPEVs.

94 new strains were isolated from humans and 18 from non-human primates (Table 2.1). Of the human strains, the most common new genotypes reported were, in order, Enterovirus species B (26 strains representing 18 types); species C (33 strains representing 11 types); A (28 strains representing 8 types); and D (7 strains represent two types).

The 18 non-human primate strains were: Enterovirus species A (9 strains

representing three types); B (three strains representing three types); and D (6 strains representing two types).

In terms of the epidemiological features, most of the newly identified NPEVs were found in sporadic cases in tropical and some subtropical regions, while a few genotypes were found in non-human primates. The non-human primate samples were identified through a single research project [100–102].

Table 2.1: Isolates and count of newly-emerged NPEVs by species.

Humans	
Type	**Count of type**
EV-A114	1
EV-A119	1
EV-A120	1
EV-A121	1
EV-A76	13
EV-A89	2
EV-A90	7
EV-A91	2
EV-B100	1
EV-B101	1
EV-B106	1
EV-B107	1
EV-B111	1
EV-B79	2
EV-B80	2
EV-B81	1
EV-B82	2
EV-B83	1
EV-B84	1
EV-B85	1
EV-B86	2
EV-B87	1
EV-B88	1
EV-B93	1
EV-B97	5
EV-B98	1

Table 2.1 Isolates and count of newly-emerged NPEVs by species and host. - *Continued from previous page*

Humans	
Type	**Count of type**
EV-C102	1
EV-C104	6
EV-C105	2
EV-C109	1
EV-C113	3
EV-C116	1
EV-C117	1
EV-C118	2
EV-C95	1
EV-C96	9
EV-C99	6
EV-D111	1
EV-D94	6
Grand Total Humans	**94**
Non-human primates	
Type	**Count of type**
EV-A119	5
EV-A89	3
EV-A92	1
EV-B110	1
EV-B112	1
EV-B113	1
EV-D111	1
EV-D120	5
Grand Total Non-human primates	**18**

Publication timeline

The timeline of newly-identified NPEV isolated and published is shown in figure 2.2. The largest number of new isolates was in 2000 whereas the largest number of new publications was 2007. Where data were available, the median number of years elapsed from isolation to publication was 5 (IQR 4-7, n=98). The detection and the reporting of some isolates could be a long delay [100].

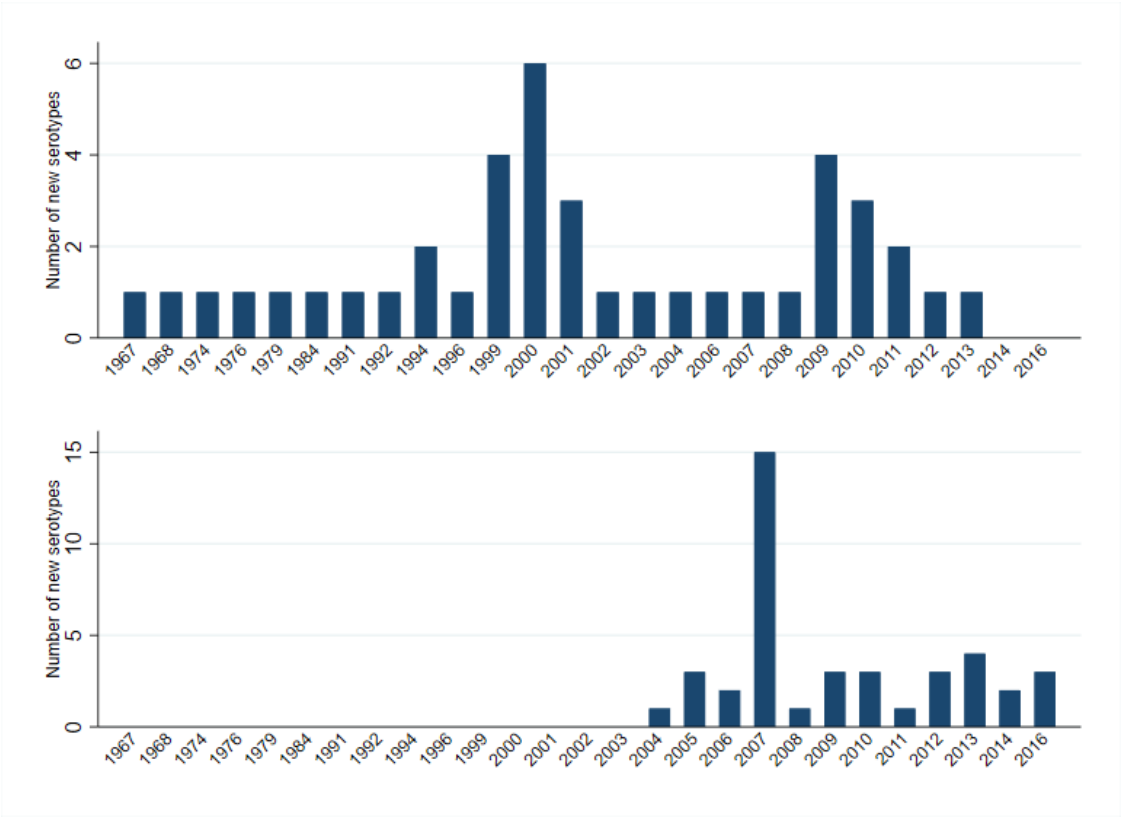


Figure 2.2: Bar chart of the number of newly-identified NPEVs isolated and data published by year.

Bar chart of the number of newly-identified NPEVs isolated and data published by year (n=41). Top: The number of newly identified NPEVs by year of isolation. Since 1999, molecular typing method has been introduced to improve characterisation of untypeable NPEVs [92, 103]. Bottom: The number of NPEVs by year of publication.

Geographic distribution

Of all the included isolates, only 107 (96%) provided detailed geographical information. Figure 2.3A majority of the newly-identified NPEVs was isolated in human, excepted for 8 types were found in free-ranging non-human primates. A map showing the geographic distribution and the composition of the newly-identified NPEVs is presented in figure 2.3.

Epidemiology

Sixty percent of human isolates in which a clinical source was reported were obtained through acute flaccid paralysis surveillance (49/80). Only six were detected through investigation of diarrheal disease and nine through investigation of a respiratory illness. 77% of the isolates (86/112) were obtained from a stool specimen. Only 28% isolates indicated age or an age band (23/80). The reported age of 11 isolates ranged from 1 month to 4 years, while other isolates were obtained from children. To our knowledge, none of the 44 new genotypes reported between 2005 and 2015 have been associated with outbreaks of illness.

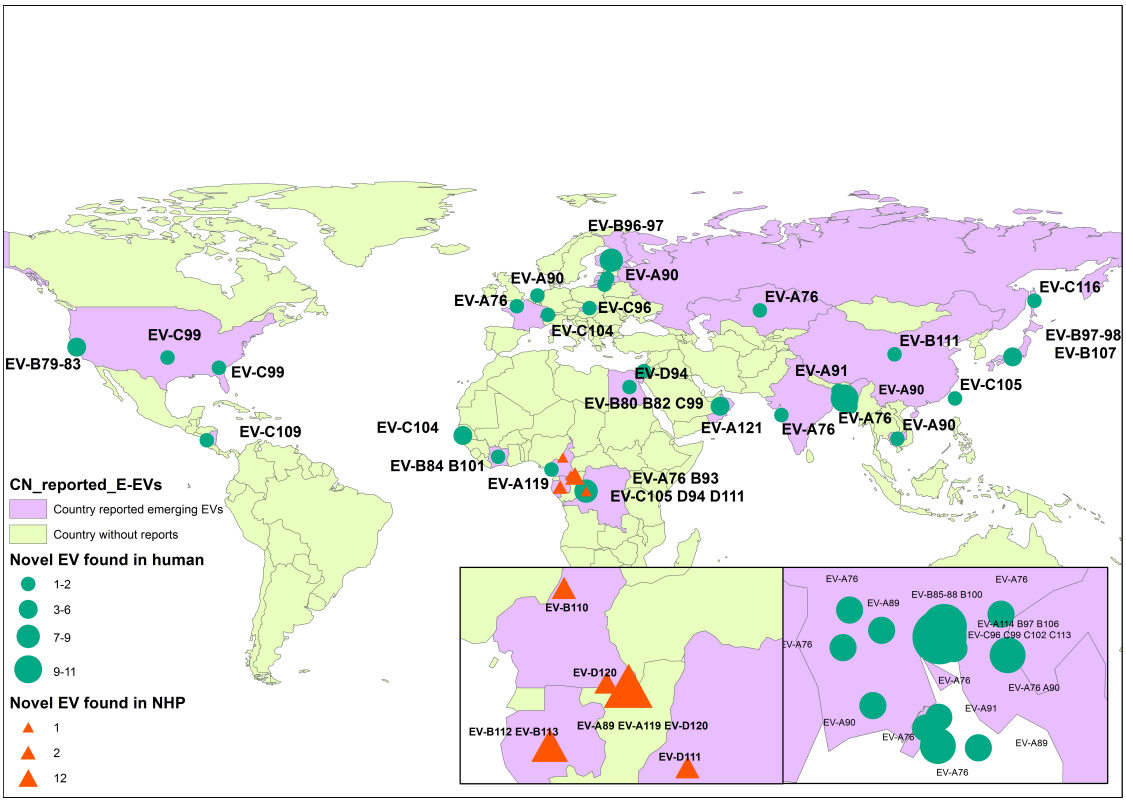


Figure 2.3: Mapping the distribution of newly-identified NPEV after 2005. Map indicating the sampling locations of the newly-identified NPEVs reported in the included studies after 2005. Human cases are indicated in green, and non-human primate samples are indicated in red. Where the size of the symbol is proportional to the number of samples from a given country.

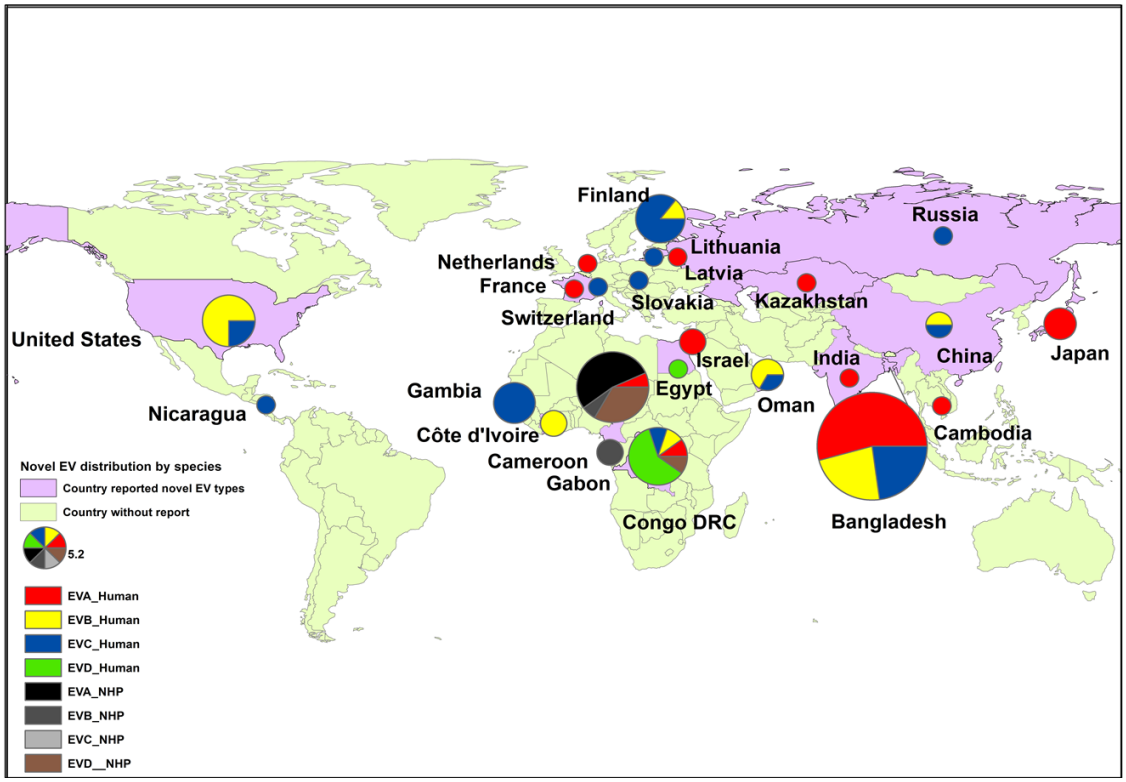


Figure 2.4: Mapping the distribution of newly-identified NPEVs. Sampling locations for the newly-identified NPEV strains on a global scale. Pie charts show detection frequencies of the newly-identified NPEV strains in each species. The size of each pie chart to the sample size in each country. Different colour shades indicate human and non-human host by EV species.

2.8 Discussion

It is generally acknowledged that NPEVs is a large group of viruses that are continuously evolving [52, 104, 105]. However, there has been few studies characterising the newly-emerged genotypes of NPEVs. This work highlights how new genotypes of NPEVs are continually being identified, mostly through the application of PCR based detection of the VP1 capsid protein gene in stool samples from patients with suspected AFP. However, these new types have not, to our knowledge, been associated with new clinical syndromes or large outbreaks of neurological or respiratory disease. Novel NPEVs have also been detected in non-human primates, with evidence of cross-over to humans, but the public health importance of zoonotic

NPEVs is unclear [106–108].

The vast genetic diversity of NPEVs is an important characteristic of these viruses. The results showed 112 separate isolates representing 44 different genotypes of NPEVs were continually being identified, constituting around 40% of all the existing NPEV genotypes. Of these, 18 and 11 NPEVs genotypes in *Enterovirus B* and *C* species consist of the majority of the newly-emerged genotypes. Two driving forces for this diversity coexist: the high error-rate in replication and the occurrence of recombination events. Previous studies reported a high average substitution mutation rate for NPEV genotypes (approximately 10^{-2} nucleotide substitutions per site per year) [109, 110]. This can result in the multitude of related, but non-identical viral variants, known as quasispecies. This high level of genetic diversity thereby lead to a great variety of amino acid sequences of the capsid region, which can explain the large number of different recognised NPEVs.

By contrast, recombination events occur very frequently and participate extensively in the genetic diversity of these viruses [104, 111]. Contemporary recombination events have been observed within the same species in regions encoding the non-structural proteins, indicated by the incongruency between phylogenetic groupings of individual genotypes in these genome regions [100, 102, 111, 112]. Such recombination has also been recognised as a driving force for the genetic diversity and the evolution of NPEVs [104, 111]. Similarly, a large-scale genomic analysis of 2949 complete genomes of NPEVs suggested that frequent recombination events occurred in NPEV evolution. The P2 and 2A region of the genomes in particular were identified as a recombination hotspot in *Enterovirus B* and *C* species [104]. The study also reported 25% of recombined sequence fragments did not closely resemble known NPEV sequences in the publicly available databases, which indicated the existence of a large genetic reservoir in the environment that has potential to fuel the combination and accelerate the emergence of new NPEV genotypes [109].

The genetic diversity also explains a substantial part of the variety of their associated clinical syndromes. A wide range of clinical syndromes was reported among human infections associated with these newly-emerged NPEVs, with AFP as the most common disease syndrome. Compared with the genotypes reported before 2005, the disease spectrum of the different newly-emerged genotypes overlap considerably [26, 113]. Young children of less than 5 years accounted for about 50% of the individuals with NPEV isolates, largely due to the worldwide surveillance for AFP [114]. These findings are consistent with previous observations of disease syndromes [26, 113]. The majority of newly-emerged NPEV genotypes were detected in stool samples, suggested their enteric tropism [115].

Eighteen isolates representing 8 genotypes were obtained from non-human primates, of which EV-A89 and EV-D111 were reported in both humans and non-human primates. With multiple genotypes, a high mutation rate, and frequent recombination events, NPEVs may have potential for cross-species transmission [102, 106]. A recent phylogenetic analysis of EV-D111 strains isolated in samples from both human and non-human primates reported a clustering of these strains within species D, with a high bootstrap value of 100%. The pairwise comparisons showed their nucleotide identity was more than 88-99% based on complete genome sequences, indicating the existence of a single lineage [115]. Similarly, a systematic review also provided phylogenetic and serologic evidence on the zoonosis potential of a wider genus of these viruses [106].This suggested the zoonotic potential of these viruses could not be ruled out.

Restricted by using the conventional methods for diagnosis and typing, a long delay has been observed in detection and reporting these newly-emerged NPEVs [116]. The discovery of these new NPEV genotypes underscores the importance of genome sequencing and phylogenetic reconstruction techniques in identifying aetiologies. Complete genome or different combinations of sequence fragments were used for genotyping [101]. In particular, VP1 is the necessary genome region when

assigning new types [104]. This is supported by the fact that phylogenetic grouping in this region usually linked to serotypes, because VP1 is considered as the protein region that usually does not recombine [100, 102, 104, 111, 112].

Phylogenetic analyses suggested that most of the newly reported EVs are genetically distinct from all the previously known EVs, determined by genomic sequences less than 75% identical (<85% amino acid identity) in complete or partial VP1 sequence [101]. But it appeared that this 'gold standard' may not be universally applicable [109, 117]. In pairwise sequence comparisons, overlap in percentage identity between comparisons of homotypic strains and heterotypic strains were reported, largely due to sequence diversity [117, 118].

The identification of newly-emerged NPEVs has been largely based on poliovirus and environmental (sewage) enterovirus monitoring, with a general absence of wider surveillance [52]. More recently the European Non-Polio Enterovirus Network (ENPEN) has been established and provided recommendations on enterovirus diagnosis and characterisation [14]. In particular, NPEN recommend that respiratory, stool, CSF and blood samples are submitted from patients with suspected neurological infections for EV testing using RT-PCR assays targeting the 5' non-coding region. In addition, sequencing of the VP1 gene is recommended for typing, with additional sequencing if a new recombinant form of EV is suspected. Despite the difficulties, ENPEN recommend that viral culture capabilities are maintained and used for further characterisation of potentially novel or worrisome strains. Notably, we found that for the majority of the newly reported NPEVs, the genome sequences were not published within 4 years. Efforts are needed to improve the availability and completeness of data on NPEV detection and characterisation.

There are several limitations in this study. The review was not undertaken as a formal systematic review with a full protocol for data extraction and evidence grading. However, the review was not designed to appraise the strength of evidence related to a particular question and it was not anticipated that the review would

generate numerical data such as prevalence rates, burden of disease or effect size estimates. Therefore, a formal data extraction and statistical analysis plan was not considered to be required. Most studies included in this work were based on phylogenetic or virologic analyses of selected NPEV isolates and some studies had a retrospective analysis of the clinical samples with small sample sizes. These characteristics restricted the representativeness of the reported data for age of patients, level of circulation, and geographical region. The scarcity of testing for type-specific enterovirus also provided a bias for data interpretation [52]. Additionally, the documentation here of the newly-emerged NPEV is not exhaustive, since a timeframe of 2005 to 2015 was imposed. Due to the high volume of literature reporting NPEVs, a more systematic literature review with characteristic description and quality appraisal should be performed with two independent reviewers. GIDEON, a bibliographic database supporting medical decision for clinicians, was searched but yielded few eligible study. This is because enterovirus infections in human is so common that sporadic cases caused by newly emerged NPEVs are unlikely to be recognised epidemiologically or detected by commercial diagnostic assay.

The results should be interpreted with caution. Each of these isolates presents only a temporal and geographic snapshot in the evolution of NPEVs that may or may not be representative of their particular serotypes or of enteroviruses as a whole. Generalizable patterns may be discerned only when careful analysis of a large number of sequences are obtained from a wider temporal and geographic distribution [119].

2.9 Conclusion

This chapter provides a brief overview of the diversity, geography and source of newly-emerged NPEVs in the period 2005-2015. The results show how genetically distinct NPEVs were isolated, detected and reported since the introduction of molecular diagnostic methods and reflect the dynamic and evolving nature of this

large group of viruses. Most were identified through AFP surveillance with, notably, none associated with disease outbreaks. The wide range of clinical syndromes was consistent with previous studies on NPEV associated diseases. Extra-neurological complications (respiratory illness) are of particular concern in future outbreaks. Cross-species transmission of newly-identified NPEVs between human and non-human primates has occurred, indicating the zoonotic potential of NPEVs.

This work also reported that these NPEVs were detected in a diverse range of contexts and geographical locations that were associated with the availability of enterovirus research groups or surveillance network. Because of the sparse data, the extent of the circulation of the newly-identified NPEVs remained unclear. Perceived barriers to NPEV detection included time lag between isolation and reporting and the use of in-house diagnostic assays and sequencing.

A particular feature is the scarcity of data associated with new isolates. To improve the diagnostic preparedness, a global surveillance network for NPEVs, together with simple and rapid diagnostic tests, is needed to monitor the trend or circulation of symptomatic infections in the long run. As the amount of genomic data rises, the relationship between genomic diversity and viral type-specific antigenicity should be studied.

3

The development and application of a non-polio enterovirus risk assessment tool

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3.1 Preface to the chapter

This chapter describes mixed-methods research to characterise the relative public health risk of non-polio enteroviruses (NPEVs) and identify gaps in research and development preparedness. The work in this chapter meets objective 2 of the thesis: to develop a transparent, systematic and replicable algorithm to assess the public health risk of NPEVs.

3.2 Author's contribution

I designed the study and reviewed the literature to assess the applicability of existing risk assessment tools, and selected a framework for the NPEV risk assessment tool and other prioritisation and validation approaches. I performed literature searches for all NPEV serotypes to generate a short list of NPEVs for validation. I designed three Delphi surveys to seek expert opinion on the tool, to refine and amend the tool, and to undertake the initial risk assessment by applying the tool. I was responsible for all the surveys and databases, and cleaned the data for interim and final analyses. I performed statistical analyses for all interim and final outcomes. I finalised the validated tool based on the opinion from all the experts who participated the Delphi surveys. I summarised the finalised qualitative outcome on research gaps and priorities. I drafted all the text and produced all figures and tables.

3.3 Abstract

Enterovirus is a large and ubiquitous virus genus which causes widespread infection in humans. The high transmissibility, high genetic and antigenic diversity, and

relative lack of cross-protective immunity between serotypes means that NPEVs pose an epidemic and pandemic threat. In particular, the high frequency of recombination, including the insertion of gene segments from previously unidentified viruses, suggest a high potential for the emergence of novel subtypes [104]. Given the large size of the genus (approximately 75 enterovirus serotypes among more than 300 genetic subtypes based on phylogenetic clustering), there is a need for a method to assess the comparative risk posed by different NPEVs in order to prioritise further research and development work. This work aims to develop and apply a systematic, transparent, and replicable tool for assessing the epidemic and pandemic risk of existing and emerging NPEVs. The tool was developed using a multi-criteria decision framework and validated through a Delphi process, whereby expert opinion was used to amend and refine elements of the tool. An initial risk assessment of selected NPEVs was performed through application of the tool by experts. A risk score was assigned to each element by using the scoring criteria. A weight was assigned to an element according to their varying importance. An overall risk score was derived from the risk score and the corresponding weight. In total, 122 respondents were invited to take part in the first round of the Delphi survey and 140 respondents in the second and final round of Delphi surveys. Of these, 17 (14%) participated in the first round; 39 (28%) participated in the second round; and 35 (25%) participated in the initial risk assessment by applying the validated tool in the third round. Respondents represented multiple disciplines from 24 countries in Asia, Australia, Europe, America and Africa. Overall, four core risk elements and three supplementary risk elements were included in the risk assessment tool. All the risk elements met the predetermined threshold of inclusion. The results indicated the ERAT have good reliability and validity as a risk assessment tool for NPEVs. The application of the tool yielded a prioritised list of 15 NPEVs, with the highest public health risk assigned to EV-A71 (overall weighted risk score=1.9543, overall unweighted risk score=11.0160) and EV-D68 (overall weighted risk score=1.9510, overall unweighted risk score=11.6992). The study also identified key areas where NPEV-related research is needed. Intermittent

reapplication of the risk assessment tool will be required to ensure that the priority list is aligned with the evolution and emergence of NPEVs.

3.4 General Background

In the past, waves of epidemic preparedness activity have corresponded with emerging epidemic threats. The concept of preparedness first emerged as a national biodefence issue in the 1950s in the United States [120, 121]. In the early 2000s the global spread of Severe Acute Respiratory Syndrome (SARS) was followed by increased efforts to enhance regional and global preparedness. Albeit effective, the response to the 2002-2003 SARS epidemic was hindered by confusion in the identification of the pathogen and uncertainty about the transmission route [122]. Coordinated surveillance efforts were acknowledged as a cornerstone of outbreak recognition and containment [123]. Shortly afterwards, in 2004, the re-emergence of avian influenza subtype A/H5N1 that caused severe influenza and deaths in human heightened concerns that a respiratory viral pandemic was a threat to global health security. The 2009 pandemic caused by (H1N1)pdm09 tested the national preparedness and response activities. It was found many countries experienced gaps in pandemic preparedness when confronted with a pandemic that was characterised by relatively mild clinical symptoms, with inflexible planning assumptions and difficult communications and logistic issues on vaccine and healthcare services. Risk assessment was identified as one of the key areas for improving pandemic planning and response on evidence base [124, 125]. Therefore, in the first decade of the 21st Century the outbreaks of SARS, A/H5N1 and (H1N1)pdm09 stimulated global preparedness efforts. These efforts were however mainly focused on strengthening disease surveillance and public health responses, with calls for increased multi-sectoral and cross-cutting collaboration towards disease surveillance, detection, reporting and response [126], and stockpiling and preparing for the deployment of existing resources such as antiviral agents and vaccines [127, 128].

The 2013-2016 West Africa Ebola outbreaks once again demonstrated deficiencies in disease surveillance and response, but for the first time revealed a failure of research and development (R&D) pipelines for diagnostics, drugs and vaccines (generically called 'medical countermeasures') [120, 129, 130]. In no previous outbreak had resources from diverse researchers and stakeholders been as focused on using and evaluating potential new diagnostics, treatments and vaccines [131, 132]. In the wake of the West Africa Ebola outbreak, evidence has been compiled to show the unacceptable time lag between the occurrence of an outbreak and its identification and the initiation of clinical trials [129, 133]. Subsequently, the years following the West Africa Ebola outbreak have witnessed major reforms and improvements in R&D for epidemic-prone infectious diseases [129].

WHO have accepted that R&D for medical countermeasures should be facilitated and implemented as an integral part of the preparedness for and response to public health emergencies [130]. Since 2016, efforts to strengthen the research and development preparedness and response capacity for severe epidemic-prone infectious diseases have been made through the WHO research and development Blueprint for action to prevent epidemics (R&D Blueprint) [89, 90]. The R&D Blueprint has produced a list of diseases which should be prioritised for research and development. This list is updated annually and in 2018 the prioritisation panel considered the inclusion of emergent non-polio enteroviruses. Although NPEVs were not added to the main list, the expert panel concluded that "They should be watched carefully and considered again at the next annual review. Efforts in the interim to understand and mitigate them are encouraged." [90].

3.5 Rationale for developing a NPEV risk assessment tool

Efforts to 'understand and mitigate' the epidemic risk of NPEVs is complicated by the fact that it is a large and diverse group of viruses and, apart from several commonly seen NPEVs such as EV-A71 and CV-A16, the majority of NPEVs are not well characterised. NPEVs are rapidly evolving; 44 genetically and immunologically novel NPEVs have been identified since 2005 [3]. However, the only medical countermeasure currently available is the EV-A71 monovalent vaccine licensed in China [66]. In the 2018 WHO annual review of prioritised disease, emerging NPEVs were identified as pathogens that pose major public health risk without major control initiatives, extensive R&D pipelines, or existing funding streams, or established regulatory pathways [90]. Recalling the EV-A71 outbreaks in the early 2000s and the 2014 EV-D68 outbreaks, preparedness for future NPEVs epidemics needs to be improved.

In order to identify R&D and preparedness priorities for NPEVs, it may first be useful to identify those viruses that pose the greatest risk to human health and for which R&D is currently inadequate. A structured approach to prioritising infectious pathogens for surveillance and R&D has been used before, most notably for zoonotic influenza, where, similarly to NPEVs, there is a large diversity of viruses and a need to focus limited R&D resources.

A range of methodologies, both quantitative and qualitative, have been employed to develop risk-ranking tools for prioritising infectious diseases [134]. A combination of disability-adjusted life years and current public health interest have been used to prioritise infectious diseases for mapping [135]. Cox *et al* used the Hirsch index (h-index)to rank 651 diseases and identify a list of 6 infectious pathogens with emergence concern out of more than 3000 pathogens [136]. But quantitative methods are less applicable to diseases without effective surveillance, abundant

data, spatial representativeness and heterogeneity in distribution [137]. WHO used a Delphi technique to prioritise a list of 20 infectious diseases for surveillance in the South Eastern Europe [138]. Humblet *et al* used a multicriteria decision analysis (MCDA) procedure to identify 20 zoonoses with high importance [139]. The United States CDC developed an influenza virologic risk assessment tool (IRAT) to prioritise emerging influenza viruses for research and vaccine development [140, 141]. WHO developed the Tool for Influenza Pandemic Risk Assessment (TIPRA) to inform decision-making for influenza viruses with pandemic potential [142].

Many risk-ranking methods share two common elements: 1) explicit 'criteria' that are used to assess risk, for example, in the TIPRA an influenza virus type was assigned low risk for risk element 'disease severity' according to the corresponding 'criteria' if it only causes uncomplicated illness or mild signs and symptoms [142]; 2) the application of 'weighting' so that greater or lesser influence can be apportioned to each risk criteria based on expert opinion of their relative importance. [143].

To better evaluate the relative pandemic threat of NPEVs, this work aimed to develop, validate, and test a tool for prioritising NPEVs for future research and development.

3.6 Aim and objectives

The overarching aim of this work is to assess the relative public health risk posed by different NPEVs. The first objective of the work was to review existing pathogen prioritisation tools and to assess their applicability to NPEVs. The second objective of the work was the development of a systematic, transparent and replicable tool to identify the NPEVs that pose the greatest public health risk. The third objective of the work was the application of the tool to yield a prioritised shortlist of NPEVs and the elicitation of future research and development priorities.

3.7 Materials and methods

A range of research methods were adopted in this work. The results in each stage were used to inform the selection of the methods in the next stage. An overview of the study process is shown in figure 3.1.

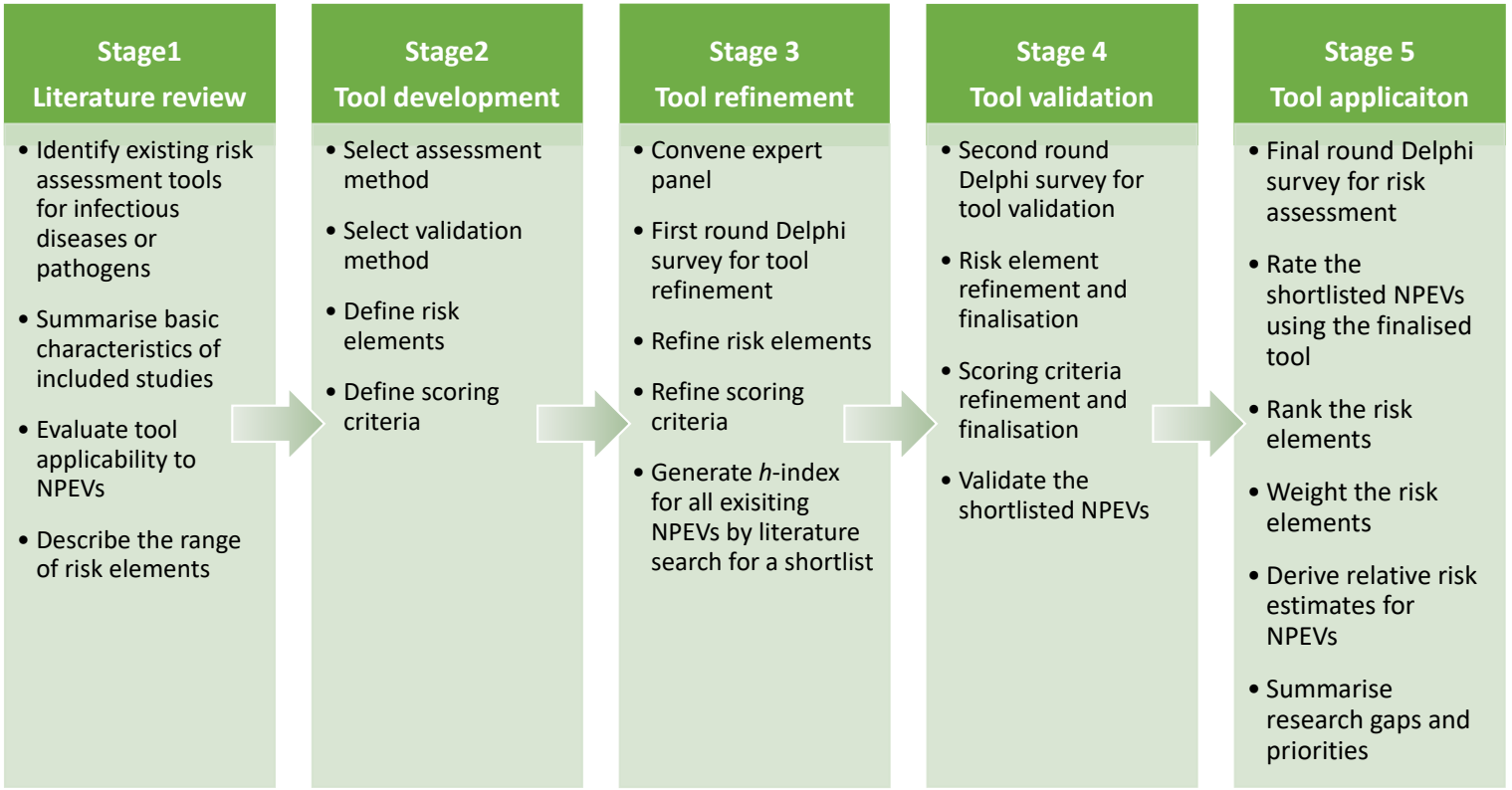


Figure 3.1: Schematic overview of the study design and objectives in each stage.

3.7.1 Literature review of existing risk assessment tools

Search strategy

To identify existing risk assessment tools a literature search was performed to retrieve eligible studies. MEDLINE (PubMed interface, 1950 onwards) was searched from inception to August 2016 using medical subject headings (MeSH) and free text. The search term was as follows: ((infectious disease* OR emerging infectious disease*) AND (epidemic* OR pandemic*)) AND ((risk assessment [MeSH Major Topic]) OR (risk assessment tool OR prioritisation tool OR risk-ranking) OR (prioritis* OR risk assess*)). The search was supplemented by hand-searching of grey literature in Google Scholar and Public Health England database. The reference lists of all included studies were reviewed to identify any additional relevant publications. No language restriction was imposed. The title and abstract of all articles retrieved through the search were reviewed twice by a single author to identify potentially relevant studies for which the full text was to be retrieved and reviewed to determine eligibility.

Study selection

A study was included if it met all of the following criteria: (1) the targeted scope of prioritisation involved NPEVs or a group of human infectious diseases or pathogens associated with human infectious diseases or zoonoses; (2) the outcome measures were risk, impact, or disease burden of infectious diseases or pathogens; (3) the study design was focused on risk prioritisation, risk-ranking or risk assessment.

A study was excluded if it met any of the following exclusion criteria: (1) the scope of prioritisation was not infectious diseases; (2) prioritisation scope included risk of individual patients or risk in vectors; (3) had incomplete methodologies or no description of the tool; (4) included methods only; (5) had unclear criteria for

risk assessment; (6) outcome measured was not risk, impact or burden of infectious diseases or pathogens; (7) duplicate citations; (8) study types such as reviews, epidemiological studies, animal studies, in vitro studies, case reports, case series and letters; (9) full text could not be retrieved.

Data collection

For all eligible publications, the following details were extracted: first author or institution, year of publication, prioritisation method, method to pre-select targeted pathogens or diseases, number of identified criteria for risk assessment, name of the criteria for risk assessment, method to validate criteria, method of risk assessment, weighting method, prioritisation scope and output.

Criteria for applicability evaluation

The suitability and applicability to NPEVs of the criteria used in the included studies was appraised if the study had clear scoring criteria and/or definition of criteria for interpretation. The definition of the risk element and/or the corresponding scoring criteria was evaluated. To assess if a criterion was potentially suitable for characterising the public health risk of NPEVs, the following aspects were considered: (1) if the criteria only included quantitative definition or thresholds, were the thresholds applicable to NPEV evidence; (2) if the criteria included qualitative proxy measure, whether it had analogy or homology to NPEVs; (3) was it a direct proxy measure related to NPEV infections; (4) was it biologically plausible; (5) was there any experimental evidence supporting the mechanism of NPEV infection.

3.7.2 Development of a NPEV risk assessment tool

This section describes the selection of the methods and the contents of the risk assessment tool informed by the results of the literature review.

Selection of the risk assessment method

Two methods, multi-criteria decision analysis (MCDA) and qualitative algorithm, were considered for the design an NPEV risk assessment tool. MCDA is a well-established methodological process that allows for quantification of benefits or risks by considering an explicit set of criteria in decision making [144, 145]. The MCDA process to assess the overall risk of the targets contains four main steps: (a) identifying all elements related to the risk; (b) defining the possible scoring criteria for the element; (c) scoring the element using the criteria; (d) weighting all elements according to importance and calculating the overall weighted scores based on the element weights and element scores [134, 145]. Qualitative algorithm has a flowchart-like tree structure and contains a series of decision questions with binary yes or no options, with a stopping point aligning with a level of risk [146]. After comparing the two methods, MCDA was selected as the framework of the risk assessment tool for the following reasons: (1) the multiple-criteria design of MCDA allows for the inclusion of multiple key factors affecting the spread and impact of NPEVs and yields numerical results; (2) qualitative algorithm is a tool designed for a rapid initial assessment instead of a full risk assessment and yields categorical or qualitative results.

Selection of the validation method

The validation method was based on the recommendation of the European Centre For Disease Prevention and Control (ECDC) [134, 146]. As the discrepancies

between NPEV relative risks were expected to arise out of data availability and geographic variation of NPEV impact, we selected a Delphi technique to seek opinion among NPEV experts to generate consensus. The Delphi technique is a widely used and accepted method for consensus building by gathering information from the research experience and knowledge of the participants [147]. This group communication process can be achieved by face-to-face or telephone interviews or a series of structured survey questionnaires. We decided to use online survey to seek expert opinion because face-to-face consultation was less feasible in the global context of the work.

Eligibility criteria for risk elements and scoring criteria

In terms of the framework, the tool was developed by firstly defining specific attributes that can be used to characterise public health risk. The framework of the risk assessment tool is shown in figure 3.2. These attributes were termed 'risk elements' [140, 145, 146]. For inclusion, a risk element should meet the following criteria: (1) the criteria should allow evidence-based risk assesment; (2) each element should be independent of the other elements; (3) each element should allow comparison between different serotypes; (4) each element should be amenable to quantitative or qualitative analysis; (5) each element should not be redundant with other elements [140, 142, 146]. Scoring criteria of three levels were then developed for each risk element to allow quantification of the risk associated with that characteristic [140, 145, 146]. The scoring criteria should meet the following criteria: (1) the criteria should reflect research progress in each risk element and allow for evidence-based risk assessment; (2) criteria should be mutually exclusive; (3) each criterion should be explicit enough for participant to interpret. Based on the data availability and pragmatic considerations that took into account clinical severity, geographic spread, prevention and treatment, risk elements were classified into 'core risk elements' and 'supplementary risk elements', which were systematically assessed based on a scale of 1 to 3 and -1 to 1, respectively (see results for more details).

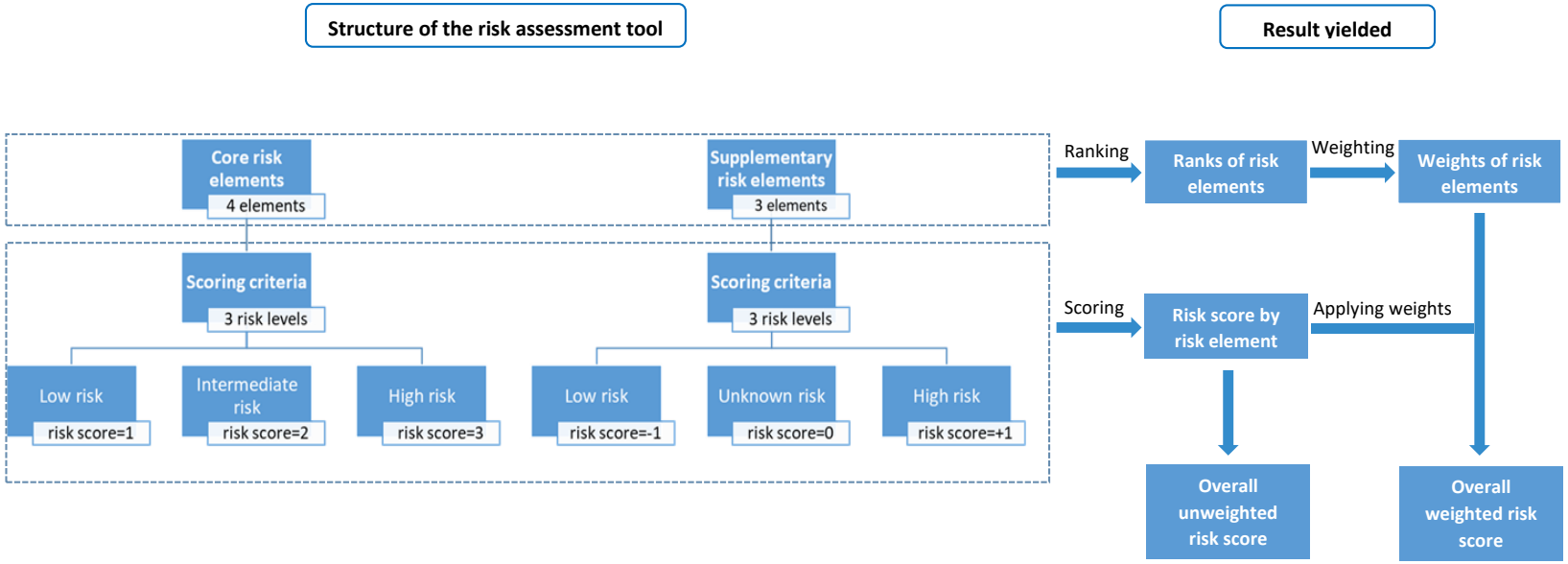


Figure 3.2: Schematic overview of the enterovirus risk assessment tool.

3.7.3 Refinement, validation and application of the tool using Delphi surveys

Expert panel for Delphi surveys

A Delphi process was used to seek expert opinion to amend and validate the tool and to perform the risk assessment in stage 3, 4, and 5. An expert panel for the Delphi survey was identified from the list of participants at the 1st international HFMD conference and from the literature search. Members were selected to try to achieve global coverage and varied subject expertise by using non-probability sampling method to choose participants who had experience in NPEVs [148]. We also accepted recommendations by members of the expert panel of other investigators to approach for inclusion in the panel. The Delphi surveys were designed and circulated using Lime Survey (version 3.6).

Questionnaire structure of the first round Delphi survey

During the Delphi consultation process, the proposed risk assessment tool was reviewed and validated.

The first round Delphi survey was composed of 33 questions. Fourteen multiple choice questions were asked to seek opinions on the draft of risk elements and the draft scoring criteria. Respondents were able to select 'unchange (agree with the inclusion and definition)', 'modify (agree with the inclusion but disagree with the definition)' or 'delete (disagree with the inclusion)'. Text boxes were provided for a written justification for the option chosen by the respondent. When modification was suggested, 14 open-ended questions (questions without pre-coded response options) were asked to elicit redefinition of risk elements and scoring criteria. The remaining questions asked about additional risk elements, suggestions on study design, and the demographics of the respondents.

Generation of a shortlist of NPEVs

Since over 100 NPEV types/75 serotypes have been identified, performing a full risk assessment to all NPEVs would be too resource-intensive and time-consuming [149]. We therefore sought to identify a shortlist of NPEVs against which the risk assessment tool could be applied. This was achieved by using the *h*-index to develop a shortlist of NPEVs. The *h*-index is a quantitative metric based on analysis of publication data using publications and citations to provide an estimate of the importance, significance, and broad impact of a scientist's cumulative research productivity. For any group of keywords or phrases, it measures the highest number of published papers, N, that have been cited N or more times. To identify the most important NPEVs based on their global impact, an attempt was made to estimate the relative importance of individual NPEV by generating h-index [150]. All known NPEVs serotypes were identified from the ICTV website (https://talk.ictvonline.org/). Bibliometric searches were conducted for using Medical Subject Headings (MeSH) and free text that included the scientific names of individual NPEV serotypes, any alternative names, acronyms, synonyms or abbreviations in accordance with National Centre for Biotechnology Information (NCBI) taxonomy website. The *h*-index of each NPEV was yielded by performing systematic searches in Web of Science covering the literature from 2010 to August 2017. A shortlist of NPEVs was created by placing the NPEV in descending order of their*h*-index and selecting the top 20 NPEVs for inclusion.

Questionnaire structure of the second round Delphi survey

In the second round, experts were asked to review a summary of the initial responses and to validate the revised tool by providing an opinion on the revised risk elements and scoring criteria. The second round of the Delphi survey was composed of 40 questions. Fourteen multiple choice questions asked about the level of agreement with the revised definition of risk elements and scoring criteria, with options to select

'unchange' or 'modify'. Free text boxes were provided to allow for the justification of the decision. Seven open-ended questions were asked to elicit opinions on the redefinition of scoring criteria when the respondent has suggested modification. Seven multiple choice questions were asked to quantify the degree of confidence of the expert decision based on a 5-point Likert scale (from very confident to very unconfident). Seven open-ended questions were asked to identify priorities for NPEV research and development.

In addition, the expert panel reviewed the 20 shortlisted NPEVs. To validate the shortlist, an array question that incorporated the 20 NPEVs with precoded response options was asked if each NPEV in the shortlist should be included, excluded or if they were uncertain (which was assigned a value of 1, -1 or 0, respectively). The remaining questions asked about the demographics of the respondents.

Questionnaire structure of the final round Delphi survey

In the final round, experts were asked to review the finalised tool and the final list of NPEVs before risk assessment. The final round Delphi survey was comprised of 25 questions. Seven array questions asked the panel to assess the level of public health risk of the 15 shortlisted NPEVs by applying the finalised scoring criteria. Seven rating questions asked the respondents to rate their level confidence based on a 5-point Likert scale in the risk assessments they had made. Seven open-ended questions sought the opinion of the respondents on research and development priorities that would improve their level of confidence. One ranking question asked the respondents to prioritise the relative importance of the risk elements from 1 to 7, where the most important risk element was assigned the highest value. The remaining questions were to collect respondent demographic information.

Risk element weighting

Following the selection of risk elements, weighting the risk elements is another key component. Weighting allows an additive multi-attribute model to be applied to derive an overall risk estimate for NPEVs [143]. As some risk elements are more important than others, a variety of approximation methods have been developed to estimate the 'true' weights for different risk elements. There is no agreement as to which method procudes more precise results since the 'true' weights remain unknown [151, 152]. Rank order centroid weighting method was selected because it would be easier for the expert panel to decide the ranks of risk elements rather than the exact weights and it was found more accurate than other approximation methods [151, 152]. This approach produces an estimate for weights that minimizing errors by identifying the centroid of all possible weights maintaining the rank order of risk element importance. It is under the assumption that the weights are uniformly distributed on the simplex of rank order centroid weight $w_{r1} > w_{r2} > ... > w_{rn}$, where $w_{r1} + w_{r2} + ... + w_{rn} = 1$ and r_i denotes the rank order of the ith risk element [151].

Use of such weighted average depends on the assumption of mutual independence of preferences, meaning the judged importance of preference on one element will be independent of its judged importance of preference on another. To ensure the weights should be meaningful, the approximate method was used to assign a total of 100 points to the set of weights for each element [153, 154]. For example, if a risk element was given a weight of 80, the risk score would be calculated by multiplying 0.8. The weighting process enabled the risk scores to be combined simply by adding their weighted scores [155].

Generation of the overall risk scores

Based on the additive multi-attribute model, the overall unweighted risk score (also termed crude overall risk score) and overall weighted risk scores for each NPEV

were calculated in the risk assessment. These were performed simply by adding up the (unweighted or weighted) risk scores by each risk element.

3.7.4 Statistical analysis

In each round of the surveys, for each risk element and scoring criteria the proportion of experts who responded 'unchange', 'modify' or 'delete' was calculated. To make decisions on including or excluding individual risk elements, we applied predefined threshold as follows: we would retain a risk element in the next round if $> 50\%$ of the ratings were 'unchange' and $< 25\%$ were 'delete'. We would modify the risk element if $> 50\%$ of the ratings were 'unchange' and $< 25\%$ of 'delete'. We would add or delete a suggested risk element if $> 50\%$ of responses were 'add' or 'delete' respectively.

Descriptive statistics are presented as frequencies for categorical variables and mean or medians with interquartile range for continuous variables.

Calculation of the weight of risk elements

To be succinct, for a simplex of 7 risk elements the defining vertices are $e_1 = (1, 0, ..., 0), e_2 = (1/2, 1/2, 0, ..., 0), ..., e_7 = (1/7, 1/7, ..., 1/7)$. The rank order centroid formula below was developed to approximate the centroids of weights by averaging the corresponding coordinates of the defining vertices. This ensures the greatest weight is assigned to the most important risk element. For example, seven risk elements are used in the risk assessment, and the weight for the most important risk element (i=k=1) is determined by calculating (1+1/2+1/3+...+1/7)/7, which equals 0.3704 [142].

In practice, determining weights for risk elements involved two steps: ranking of the risk elements by experts according to their relative importance, and then assigning weights according to the ranks by using the formula. This allows relative

importance generated in a more subjective way to yield exact weights in an objective way [153]. This allows for comparison of overall weighted risk scores and also ensures the weights of the risk element sum to 1 [156].

The approximation formula below (formula 4.1) illustrates the calculation of centroid weight for the ith most important risk elements. Summation sign ($\sum$) was the expression for a sum of the elements of a sequence (on the right of the summation sign). The notation at the bottom of the summation sign (the index of summation) indicated the starting point or lower limit of summation, while the notation on the top of the summation sign indicated the stopping point or upper limit of summation.

W_k is the surrogate weight of the kth element, where k corresponded to the rank of the risk element. The weights (W_k) of risk elements were calculated according to the formula below, where n presents the number of all risk elements (n=7).

$$W_k = (1/n)\sum_{i=k}^{n}(\frac{1}{i}) \tag{3.1}$$

Calculation of the overall risk score for NPEVs

Briefly, adding together the unweighted/crude risk score for each risk element calculated the overall unweighted risk score for an NPEV type, while adding up each weighted risk score calculated the overall weighted risk score for an NPEV type.

The overall risk score of the shortlisted NPEVs was calculated using a simple addictive model using the mean of each risk element, adjusted by the corresponding weight of risk elements. The overall unweighted risk score (S_{Ui}) of an NPEV type i was calculated as:

$$S_{Ui} = \sum_{j=1}^{n} X_{ij} \tag{3.2}$$

While the overall weighted risk score (S_{Ai}) of an NPEV serotype i was calculated as:

$$S_{Ai} = \sum_{j=1}^{n} W_j X_{ij} \tag{3.3}$$

where W_j is the weight of criterion j and X_{ij} is the scoring value assigned to NPEV serotype i on the criterion j.

In the final Delphi survey, the internal consistency of the risk assessment tool was quantified by the Cronbach's α, which assesses whether the risk elements reliably estimate the public health risk for each shortlisted NPEV. An α value of 0.6 and greater was considered acceptable [157].

Microsoft Excel 2016 was used for data management. STATA statistical software (version MP 15.0) and Python (version 3.6) were used for statistical analysis and graphics.

3.7.5 Qualitative analysis

Textual data for amendments and research gap analysis in all Delphi surveys were merged anonymously. A single investigator reviewed the textual data in all three surveys. In the first two rounds of the surveys, key themes for amendments or refinements in any risk elements and scoring criteria were identified by thematic analysis. In the second and the third round of the surveys, research gaps and priorities for NPEV infections were identified by thematic framework analysis.

Thematic analysis involved five main phases: familiarising with data content, generating initial codes, searching for themes, reviewing candidate themes, and

(defining and naming themes). In this study, familiarisation with the data involved reading the data and annotating the text segments potentially of interest to understand what the data mean. Generating the initial codes involved identifying repeated patterns in textual segments and assigning codes to these text segments at semantic and latent levels. For each text segment of interest, a decision on whether to establish a new code or to use an existing code was made. To ensure the existing codes better fit all the included content, they were modified or expanded to incorporate more text segments that share similarity. This process was repeated until the entire data set was fully coded and collated. The next phase included sorting different initial codes into candidate themes and exploring the relationships between codes and between themes. This process was done by collapsing or clustering codes that seem to share unifying feature to reflect a coherent pattern. Reviewing the potential themes involved checking the themes against the collated text segments and codes. Miscellaneous codes may be relocated and end up as part of new themes or be discarded. This phase ended when themes became distinctive but cohesive as a whole. Themes were then defined or named to specify the focus of the research question [158–160].

Thematic framework analysis was performed using the following phases: familiarisation with data, identifying a thematic framework, coding or indexing, and interpretation [161, 162]. Atlas.ti cloud (version beta) was used to manage coding in the analysis.

3.8 Results

3.8.1 Review of published risk assessment tools

Literature search

The search strategy identified a total of 244 publications pertaining to infectious disease risk assessment tools, which were screened based on the title and abstract. Of these, 207 studies were excluded after reviewing the titles and abstracts since these studies either did not meet the inclusion criteria (n=165) or met the exclusion criteria (n=42). The full texts of the remaining 37 studies were examined. A further 16 studies were excluded because of incomplete methods, incomplete results, unclear assessment criteria, no full-text or unsuitable study type. A total of 21 studies involving 19 distinct risk assessments or risk prioritisation exercises met the eligibility criteria and were included in the qualitative synthesis [108, 135, 136, 139, 142, 150, 163–176]. Of these, the criteria used in 16 studies (14 distinct risk assessments/ risk prioritisation exercises) were reviewed and the applicability of the criteria to NPEVs was assessed [108, 139, 142, 163–174]. The remaining 5 studies, which used quantitative methods, were included for the purposes of method selection only. A flow diagram describing the process for selecting studies is presented in figure 3.3.

Basic characteristics of included studies

Overall, the procedures for developing the risk assessment tools involved: identifying the scope of the prioritisation exercise, selecting a method for risk assessment or prioritisation, identifying the criteria for prioritisation, validating the prioritisation criteria, and selecting a method to weight the prioritisation criteria.

Specifically, 5 studies used quantitative methods (disease burden estimates or H-index), while 14 studies (12 risk prioritisation exercises) employed semi-

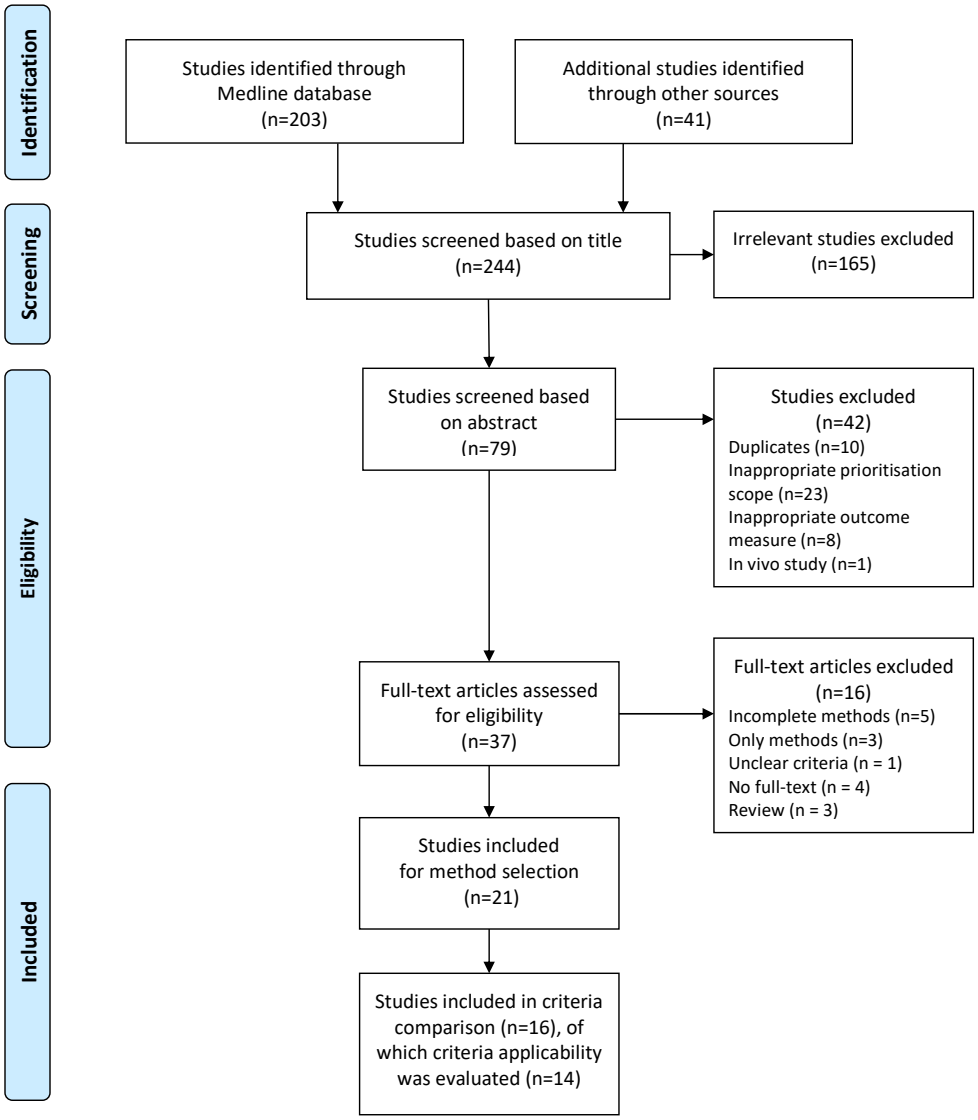


Figure 3.3: Flow diagram of search strategies and review process based on PRISMA statement. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analysis.

quantitative methods (MCDA, risk matrix or other semi-quantitative approach) and 2 studies adopted qualitative algorithms. Of all 19 risk prioritisation exercises (21 studies) [1], 58% (11/19) shortlisted the prioritisation scope. Of the 14 risk prioritisations using semi-quantitative or qualitative methods, 93% (13/14) provided a definition of the prioritisation criteria and/or the corresponding scoring values, but only 36% (5/14) validated the criteria. Weighting criteria were used in 83% (10/12) of the semi-quantitative studies. In terms of the data collection method for the risk assessment, 16% (3/19) were performed using online tools or electronic questionnaires, 21% (4/19) convened a face-to-face meeting or discussion, and 10% (2/19) used a Delphi technique. The basic characteristics of the included studies are shown in table 3.1 and 3.2.

[1]The number of the unique risk prioritisations rather than that of all included citations is used as the denominator for the following description. This is because not all citations had complete reporting of method for assessment.

Table 3.1: Basic characteristics of the included studies using qualitative and semi-quantitative methods.

Study ID [Reference ID]	Prioritisation method	Pre-selected scope of interest	Criteria identification	Criteria validation method	Criteria Weighting method	Data collection tool for assessment	Prioritisation scope	Output
Cox 2012, [165] Cox 2013 [166]	Multi-criteria decision analysis (MCDA)	NS	40 defined criteria with varied scoring scales	Discussed with experts	Weights were calculated using a Likert scale with assigned values. The other weighting method used probability distribution modelling	Electronic questionnaire	9 infectious diseases of human and animal influenced by climate	Pathogen ranking
Balabanova 2011 [167]	MCDA	5 pre-selection criteria	10 criteria with 3-tiered scoring scales	Delphi technique	Weights were calculated using a predetermined scale	Delphi technique	127 pathogens	Pathogen ranking
Cardoen 2009 [168]	Semi-quantitative approach	Literature review and expert opinion	5 defined criteria with varied scoring scales	NS	Weights were calculated by Las Vegas method using a scale of 5 scoring values	NS	51 zoonotic pathogens	Pathogen ranking
Havelaar 2010 [169]	MCDA	5 selection criteria	7 criteria with scoring values	NS	Mean weights derived by consensus were used	Expert panel discussion	86 emerging zoonotic pathogens	Pathogen ranking
Humblet 2012 [141]	MCDA	Diseases reported to World Organisation for Animal Health	57 criteria with scoring values	Reviewed by experts	Deterministic mean and probabilistic with distribution functions	NS (assessed but unclear methods)	100 animal diseases and zoonoses	Disease ranking
Morgan 2009 [170]	Qualitative algorithms	NS	12 decision questions focused on impact	NA	NA	NS	1 example given	Risk levels
Palmer 2005 [111]	Qualitative algorithms	NS	8 decision questions	NA	NA	NS	5 emerging animal disease pathogens	Risk levels
Trock 2015 (IRAT) [171]	MCDA	NS	10 defined criteria	NS	Rank centroid weight method was used	Meeting	2 virus variants	Risk scores
Garner 2015 [172]	MCDA	245 infectious diseases listed by previous work	10 defined criteria with scoring values	NS	Weights derived by consensus	Working group discussion	32 pathogens	Risk scores and risk levels
Economopoulou 2014 [173]	Risk matrix	5 selection criteria	2 composite parameters (11 criteria) with scoring values	NS	NA	Delphi survey	71 infectious diseases with pathogens	Surveillance priority level
Gilsdorf 2011 [174], Krause 2008 [175]	MCDA	6 selection criteria	12 defined criteria with scoring values	Online survey	NS	Online survey	85 selected pathogens	Prioritisation scores
McKenzie 2007 [176]	Semi-quantitative approach	A short list generated by experts	3 undefined components with scoring values	NA	NA	NS	82 selected pathogens	Risk scores
WHO TIPRA 2016 [144]	MCDA	NS	9 defined criteria with scoring values	Teleconference discussion	Rank centroid weight method was used	Meeting and electronic survey	8 influenza strains	Risk scores
Public Health England 2016 [179]	MCDA	NS	8 criteria with risk assessment results	NS	NA	NS	EV-D68	Risk category

NS= Not stated; NA= Not applicable

Table 3.2: Basic characteristics of the included studies using quantitative methods.

Study ID [Reference ID]	Prioritisation methods	Pre-selected scope of interest	Criteria identification	Risk assessment method	Assessment validation	Weighting method	Data collection method	Prioritisation scope	Output
Van Lier 2016 [177]	Quantitative estimates – disease burden	Selected based on previous project	NA	Disease burden software toolkit	NS	NA	NS	32 selected infectious diseases	Disease burden and disease ranks
Pigott 2015 [137]	Quantitative estimates – DALY and priority score	Selected in a previous review	NA	A proposed framework using global burden and disease's importance	NS	NA	NS	176 diseases	Disease ranks
McIntyre 2014 [152]	Quantitative estimates – *h* index	NS	NA	Literature search	Disease burden	NA	NS	100 human and animal pathogens	Pathogen ranking
Cox 2016 [138]	Quantitative estimates – *h* index	NS	NA	Literature search in Web of Science	*h*-index generated by literature search in PubMed	NA	NS	651 human pathogens in North America	Pathogen ranking
McIntyre 2011 [178]	Quantitative estimates – *h* index	Previously generated database	NA	Literature search in Web of Science	DALY estimations	NA	NS	1414 human pathogens	Pathogen ranking

NS= Not stated; NA= Not applicable

Comparison of criteria or risk elements, and evaluation of the applicability of the existing tools to NPEVs

The criteria applied in the semi-quantitative and qualitative prioritisation exercises were summarised (n=14) [108, 139, 142, 163–174]. Only those with definitions of criteria or scoring values were used to evaluate the applicability to NPEVs (n=9) [139, 142, 163–165, 167, 169, 170, 172, 173]. The applicability of prioritisation criteria used in 5 quantitative studies were not assessed, because the methods were disease-specific (burden or h-index) and therefore were not applicable to NPEVs [135, 136, 150, 175, 176].

The aims of disease or pathogen prioritisation in the included studies were to estimate the risk of emergence or the impact of pathogens, or to set priorities for surveillance, prevention or control infectious diseases. Correspondingly, a wide range of criteria (risk elements) were included. The most commonly used criteria were the availability of specific treatment for disease (71%, 10/14) and incidence of disease (64%, 9/14), followed by disease severity (57%, 8/14), prevention of disease (50%, 7/14), case fatality rate and the presence of a reservoir of the pathogen (both 43%, 6/14). Other criteria used included disease trend over time, transmissibility of disease between humans and animals (both 36%, 5/14), genomic characteristics, population susceptibility, and economic impact (all 29%, 4/14).

Clear definition of the criteria or a description of the scoring values were available in 9 out of 14 risk prioritisation exercises. They were included in the applicability evaluation. The median proportion of applicable criteria to NPEVs in these risk assessments was 62.5% (IQR 47.5-70.0) with the highest proportion being 83.3% and the lowest 42.1% [139, 142]. An assessment tool developed by US CDC and later adapted by WHO yielded 70.0% and 83.3% of applicable criteria, respectively. The tool was modified by Public Health England (PHE) and applied to the assessment of the risk associated with the NPEV serotype EV-D68 (personal communication - Gavin Dabrera). However the proportion of its criteria that were assessed as

applicable to NPEVs was merely 62.5%. The included criteria and the applicability evaluation are summarised in table 3.3.

Of note, none of these studies were designed specifically to evaluate the relative public health risk of NPEVs in general. In terms of prioritisation scope, only two of these studies involved NPEVs in the risk assessments [165, 177]. Balabanova *et al* suggested that enteroviruses and echoviruses were in the high priority group for surveillance and epidemiological research, while PHE assessed the epidemic risk of EV-D68 and classified it as low risk in the UK. In another risk prioritisation, it was suggested that coxsackieviruses (CVs) and enteroviruses (EVs) should be added to the prioritisation scope. These viruses were not prioritised in the study [172].

Table 3.3: Comparison of the prioritisation criteria and applicability in the included studies.

Study ID [Reference ID]	Criteria	No of criteria	No of criteria potential applicable to NPEVs	Most important criteria suggested by authors
Cox 2012 [165], Cox 2013 [166]	12 elements for disease epidemiology, 5 elements for ability to control, treat and monitor, 12 elements for influence of climate change in Canada, 8 elements for burden of disease, 3 elements for economic and social impact	40	19	Potential economic impact, severity of disease in population, case fatality rate, type of climate, incidence of human diseases, climatic conditions, presence of a vector, presence of host species, transmission mode, pathogenicity in general population
Balabanova 2011 [167]	10 criteria including disease epidemiology, disease trend, direct and indirect cost, prevalence of disease sequelae, case fatality rate, proportion of public health actions needed, public attention, prevention and treatment, etc.	10	7	Case fatality rate, prevention possibility, and proportion of public health actions required,
Cardoen 2009 [168]	5 criteria including disease severity in humans, occurrence of the disease, occurrence in live animals, disease severity in animals and economic impact, occurrence of the agent in food	5	Not assessed [a]	Disease severity in human, disease occurrence, and occurrence of agent in food
Havelaar 2010 [169]	7 criteria including probability of introduction to the country, transmission in animal, animal-human transmission, human-human transmission, morbidity, mortality, economic impact in animals	7	3	Case fatality rate, animal-human transmission
Humblet 2012 [141]	5 categories with 57 criteria including illness rate, case-fatality rate, agent specificity, mode of transmission, incubation period, clinical course, environmental persistence, epizootic potential, evolutionary characteristics of pathogen, etc.	57	24	Epizootic potential, effectiveness of prevention, loss of productivity, illness rate and impact on animal welfare and biodiversity (the most important criterion in each category)
Morgan 2009 [170]	8 decision questions including human-human transmission, zoonotic potential, population susceptibility, natural reservoir, disease severity, transmission rate in humans, case severity rate, availability of effective interventions	8	Not assessed [a]	Effective treatment, high transmission rate in human, case severity rate
Palmer 2005 [111]	8 decision questions including whether pathogen identified for animal disease, biological mechanism for causing human infection, existence of an equivalent disease in humans, diagnostic test availability, exposure by subgroup, way to identify cases, existence of possible cases, human-to-human transmission	8	Not assessed [a]	Human-to-human transmission, existence of possible cases, way to identify cases
Trock 2015 [171]	10 criteria including genomic variation, receptor-binding properties, transmissibility in animal models, antiviral treatment susceptibility, population immunity, disease severity, antigenic relationship to vaccines, human infections, infections in animals, and global distribution in animals	10	7	Disease severity, population susceptibility, human infection, treatment options
Garner 2015 [172]	10 criteria including incidence, annual mortality, case fatality, communicability, treatability, clinical impact, public/political attention, 10-year projection of incidence, economic impact, preventability	10	6	Incidence, treatability, case fatality rate
Economopoulou 2014 [173]	2 categories (including 11 criteria): public health impact and likelihood of occurrence without definition	11	Not assessed [a]	Not stated
Gilsdorf2011 [174],Krause2008 [175]	12 criteria including burden of disease, incidence, severity, mortality, outbreak potential, trend, emergence potential, validity of epidemiologic information, international duties and public attention, evidence for pathogenesis, preventability and treatability	12	9	Incidence, severity, mortality, outbreak potential, preventability
McKenzie 2007 [176]	Likelihood of spread and consequence of spread in free-living wildlife, captive wildlife, humans, livestock, and companion animals.	3	Not assessed [a]	Not stated
WHO TIPRA 2016 [144]	9 criteria including infection in animals, geographic distribution in animals, genomic characteristics, receptor binding properties, transmission in animal models, susceptibility to antiviral treatment, disease severity, human infection, population immunity, of which 6 were for impact assessment	6	5	Disease severity, population immunity, susceptibility to antiviral treatment, genomic characteristics
Public Health England 2016 [179]	8 criteria including person-to-person transmission, animal to human transmission, case severity, genomic variation (flu only), population immunity, transmissibility in animal models, intervention-antivirals, intervention-vaccines (flu only)	8	5	Person-to-person transmission, animal to human transmission, and case severity

[a] Criteria (a.k.a. risk elements) were not assessed because there was no definition and assessment could lead to subjective judgement.

3.8.2 Identification of risk elements for inclusion in the NPEV risk assessment tool

Based on the literature review, the criteria previously used to prioritise infectious diseases have generally fallen into three broad categories: a.) the attributes of the disease and the population at risk, b.) the attributes of the pathogen itself, and c.) the availability of effective vaccines or treatments.

When considering the applicability of different risk elements to NPEVs, an early issue that was identified was how to handle absence of information. Due to the diversity of NPEVs, the completeness of information may vary considerably between viruses. An absence of information cannot be interpreted as either increasing or decreasing risk i.e. absence of evidence on risk should not be confused with evidence of absence of risk. Risk elements where absence of evidence was considered likely to be a major factor were scored between minus and plus one: -1 if there was evidence that suggested risk was low, +1 if there was evidence that suggested risk was high, and zero if there was no evidence either way. These were termed 'supplementary risk elements' and all other risk elements were termed 'core risk elements'.

The first iteration of the proposed tool included 7 risk elements. A summary of the risk elements and their scoring criteria is provided in table 3.4. Of the seven risk elements, four were defined as core risk elements (disease severity, incidence and distribution, stage of vaccine development, stage of antiviral development). Whereas genetic diversity, tissue tropism and population susceptibility defined as 'supplementary risk elements'.

Attributes of disease and population

Several dimensions of potential impact were identified from the literature review which had direct applicability to NPEV, these were the frequency of severe dis-

ease, temporal trends in disease incidence, and levels of population susceptibility to infection.

Risk element 1: Disease severity This element aims to assess the severity of illness in humans following infection, or in suitable animal models as a surrogate where human infections have not occurred.

Risk element 2: Epidemiology This risk element aims to assess recent trends in disease frequency. NPEVs are associated with endemic transmission, regular annual and multi-annual outbreaks, and new large epidemics. A prime example is EV-A71 that was identified in 1969, emerged as a public health problem in the early 2000s, before becoming endemic in Southeast Asian countries with regular epidemic cycles [86, 178, 179]. This risk level associated with this risk element was determined by the patterns and trend of disease, the intensity of disease occurrence, and the geographical range [142].

Risk element 3: Population susceptibility This risk element aims to assess the extent of existing direct or cross-protective humoral immunity to a certain enterovirus genotype/serotype in the human population. Neutralising antibodies are thought to provide protection against infection, whereas T-cell mediated immunity may determine the clinical outcome following infection. The immunity responded to NPEV infections was believed to be primarily humoral [180]. Both direct and indirect effects of immunity can decrease risk of NPEV infections [66, 178, 181]. However, this is complicated by multiple factors [41]. First, the level of population immunity varied because of the waning of infection-induced immunity over time. Second, evidence indicated that there was intertypic cross-neutralising reactivity between NPEV serotypes and intratypic cross-reactivity protection against NPEV genotypes [38, 182–184]. In populations, the prevalence of neutralising antibodies

is associated with the incidence and epidemic dynamic of NPEV infections [5, 41]. Due to the uncertainty about correlates of protective immunity and likely lack of data on antibody seroprevalence for many NPEV serotypes, this domain was designed a 'supplementary risk element' with scoring of -1, 0, +1.

Countermeasures to NPEVs

Vaccines and antivirals (for prophylactic or therapeutic use) are core components of current approaches to mitigating the impact of infectious diseases. Therefore, the stage of development and availability of vaccines and antivirals were included as 'core risk elements'.

Risk element 4: Vaccine availability This risk element considers the stage of development of vaccines against NPEVs, including the availability of candidate vaccines with evidence of immunogenicity, safety and efficacy [139, 169, 170, 185, 186].

Risk element 5: Antivirals This risk element considers the stage of development of antiviral compounds and evidence of efficacy and safety for use in at-risk human populations [187].

Properties of NPEVs

Viral phenotypic traits, such as transmissibility and virulence, are co-determined by virus genotype and host susceptibility [188].

Risk element 6: Genetic diversity This risk element is intended to capture information on virus genetic diversity, which may correlate with the risk of emergence of viruses with epidemic or pandemic potential.

Genetic diversity may result in antigenic diversity and escapes both humoral and cellular population immunity [186]. Evidence shows new epidemics are often accompanied by shifts in genotype [178, 184]. Also, previous EV epidemics have been characterised by peaks of increased genetic diversity, often with genotype changes [178]. Genetic diversity may also lead to changes in tissue tropism and virulence [189]. Therefore, the risk levels associated with this risk element was determined by the evolutionary force of diversification in NPEVs [142, 169]. The availability of the evidence on genetic diversity was also considered. Evidence showed that transmission dynamics were consistent with a change in viral properties (pathogenicity, antigenicity or transmissibility) [5]. Due to likely uncertainty and lack of data on genotypic correlates of virus phenotype, this domain was designated a 'supplementary risk element' with scoring of -1, 0 or +1.

Risk element 7: Tissue tropism This risk element is intended to assess the type of tissues and cells that the virus has demonstrated capability to infect. NPEV infection is initiated after viruses bind to a receptor on the cell surface. Cellular receptors for viruses have been considered as the primary determinants of tissue tropism. NPEVs can infect a variety of target cells, including neurons, cardiomyocytes, pulmonary interstital cells, renal tubular cells and epithelial cells [180]. The replication of NPEVs in certain cells and tissues may result in a distinct pattern to each serotype [190]. Due to likely lack of data on tissue tropism for many NPEV serotypes, this domain was designated a 'supplementary risk element' with scoring of -1, 0, or +1.

Table 3.4: Draft 'risk elements' proposed risk assessment tool.

Core risk elements

		Risk score		
Risk element	**Description**	**1 (Low)**	**2 (Moderate)**	**3 (High)**
Disease severity	A measure of the severity of illness in humans following infection, or in suitable animal models as a surrogate where human infections have not occurred.	Causes almost exclusively subclinical or mild, self-limiting illness in human.	Severe illness reported only in specific at-risk groups, or evidence of severe disease in animal models of infection.	Severe illness reported in the majority of previously healthy humans.
Epidemiology	A measure of the recent emergence and the trend of disease frequency over the past 5 years.	Stable endemic or rare occurrences and stable over 5 years.	Weak or inconclusive evidence of increasing frequency and/or geographic distribution.	Clear evidence of increasing frequency and/or geographic distribution, and/or recent emergence as a cause of human disease.
Vaccine availability	A measure of the availability of candidate vaccines against enteroviruses; immunogenicity, safety and efficacy are also taken into consideration.	Vaccine with demonstrated safety and efficacy in humans.	Candidate vaccine in development but no human efficacy data.	No candidate vaccine.
Antivirals	A measure of the available antiviral compounds with evidence of efficacy and safety for use in at-risk human populations	Antiviral with efficacy and safety in observational studies and/or randomized controlled trials.	Antiviral with evidence of safety in preclinical studies.	No antiviral in the development pipeline.

Table 4.4: Draft 'risk elements' proposed for risk assessment toolrisk assessment tool - *Continued from previous page*

Supplementary risk elements

		Risk score		
Risk element	**Description**	**-1 (Low)**	**0 (Unknown)**	**+1 (High)**
Genetic diversity	A measure of the rate of genetic evolution and/or recombination of non-polio enteroviruses.	A low mutation and/or recombination rate.	Average mutation and/or recombination rate or no data.	A high mutation and/or recombination rate.
Tissue tropism	A measure of the type of tissues and cells the virus is best suited to infecting.	Lack of tropism for human respiratory, cardiac and/or neurological tissue cells.	No data / insufficient data.	Tropism for human respiratory, cardiac and/or neurological tissue cells.
Population susceptibility	A measure of the extent of cross-protective immunity to a certain enterovirus genotype/serotype in the human populations.	Widespread direct or cross-protective immunity.	No data / insufficient data.	Widespread susceptibility.

3.8.3 First round Delphi survey

Briefly, the first round of the Delphi survey sought expert opinion on the draft risk elements and scoring criteria (risk scores) (see table 3.4) and asked if, additional risk elements should be included.

Survey demographics

In total, 122 experts were invited to participate in the first round Delphi survey, of which 17 (14%) participated and returned full responses. The respondents had experience in multiple disciplines and regions [2]. By region, respondents had NPEV expertise in 7 countries in Asia (89%, 16/18) and 2 countries in Europe (2/18, 11%). The panel consisted of specialists in virology (10/32, 31%), clinical medicine (8/32, 25%), epidemiology (6/32, 19%), immunology (3/32, 9%) and Others (2/32, 6%), as well as pharmacology, vaccinology and bioinformatics (each had a proportion of 3% (1/32)).

Feedback from first round

Level of agreement with proposed risk elements and scoring criteria: The majority of respondents said that the definition of the risk elements and scoring criteria should be 'unchanged', with a median percentage of 88.2% (IQR 79.5-91.2) and 76.5% (IQR 70.6-76.5), respectively. The level of agreement with risk elements ranged from 70.6% for 'epidemiology' to 100.0% for 'antivirals'. As the level of agreement exceeded the predefined inclusion threshold (section 4.7.4; $> 50\%$ of the ratings 'unchanged') for all draft risk elements, none of the draft risk elements were excluded. By comparison, there were lower levels of agreement for the scoring criteria (from 52.9% for 'disease severity' to 82.4% for 'population susceptibility').

[2]The number of all expertise and regions is used as denominator for the following description, therefore the denominator is greater than the number of respondents.

A median percentage of 11.8% (IQR 8.9-20.6) and 23.5% (IQR 23.5-29.4) of respondents suggested revising the risk elements and scoring criteria respectively. Modification was most frequently recommended for the risk element 'disease severity' (41.2%), followed by 'epidemiology', 'genetic diversity' (both 29.4%) and 'vaccine availability' (23.5%). Experts' recommendations for amendments to the scoring criteria were as follows: 'disease severity' (41.2%); 'epidemiology' and 'genetic diversity' (both 29.4%); 'vaccine availability', 'antivirals' and 'tissue tropism' (all 23.5%); and 'population susceptibility' (11.8%). The percentage of experts' opinions on the amendment of the proposed risk elements and scoring criteria are displayed in figure 3.4.

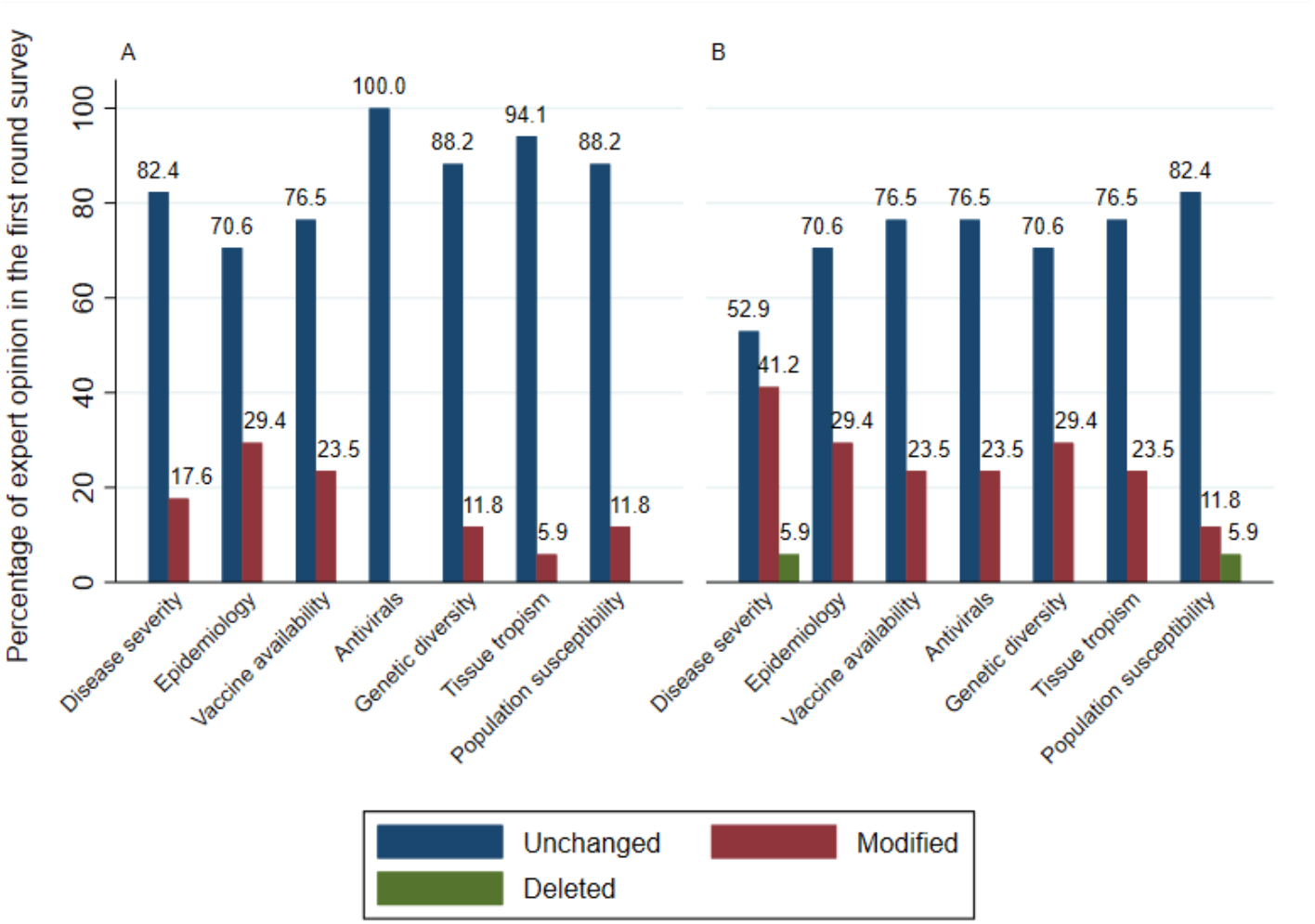


Figure 3.4: Outcome of the first round Delphi survey, expert opinion on tool amendment (n=17). (A) Bars show the percentage of expert opinion on the risk element based on a 3-tiered criteria. (B) Bars show the percentage of expert opinion on the scoring criteria based on a 3-tiered criteria.

Response to comments on core risk elements and scoring criteria: Generally, the amendments were made according to the experts' opinion in the first round. They could be categorized into three types: modifying the name of risk elements (three risk elements), the definition of risk elements (five risk elements), and the scoring criteria (five risk elements). The definition was amended by specifying indicators/features or quantitative thresholds that could differentiate the level of risks in the assessment. The scoring criteria was amended to ensure the scoring criterion remained mutually exclusive to one another and consistent to the risk assessment.

With regard to 'disease severity', key themes raised by experts included the limited evidence of a correlation between severity in animal models and in humans, and the limitations of disease surveillance, which is usually restricted to more seriously unwell cases. Correspondingly, we redefined the risk element by removing 'evidence in animal models'. In addition, to improve clarity, the scoring criterion was modified by specifying the meaning of 'severe illness', 'disease severity', excluding the term 'subclinical cases' and replacing the term 'majority' with 'a significant proportion'.

In response to the comments, we renamed the risk element 'epidemiology' to 'incidence and distribution'. We modified the definition by specifying 'disease frequency and spatio-temporal trend' as the key measures. The scoring criterion was amended to be more specific by clarifying that the epidemiological indicators of interest were: the incidence of cases, the frequency of outbreaks, and the geographical distribution.

Similarly, we renamed the risk element 'vaccine availability' to 'stage of vaccine development' based on comments that availability does not equate to accessibility and that we should capture information on candidate vaccines in the development pipeline. We modified the scoring criteria by classifying the strength of evidence from the vaccine development pipeline into three tiers: 'candidate vaccine with safety and efficacy data from at least one phase III trial'; 'candidate vaccine with

safety and efficacy data from phase II or observational studies'; and 'no candidate vaccine or a candidate vaccine with only preclinical or phase I data'.

Likewise, we renamed the risk element 'antivirals' to 'stage of antiviral development' and amended its definition correspondingly. We amended the scoring criteria by classifying the evidence by the strength of evidence to three levels: 'antiviral with demonstrated efficacy and safety data in at least one phase III clinical trials', 'antiviral with efficacy and safety data in phase II or observational studies' and 'no antiviral or candidate antiviral with preclinical or phase I study'.

Response to comments on supplementary risk elements and scoring criteria: The supplementary risk elements received fewer comments than the core risk elements. It was suggested that the term 'rate of genetic evolution' within the definition of the risk element "genetic diversity" required greater clarity for a definition of 'high and low mutation rate'. In response to the comments, we modified the risk element to focus on phylogenetic diversity rather than the absolute rates of nucleotide substitution or recombination given the association between the genetic diversity and the risk phenotypes.

We did not modify the definitions of 'tissue tropism' and 'population susceptibility'. We modified the scoring criteria for the latter by specifying the extent of 'widespread' immunity or susceptibility.

Response to comments on study design No additional risk elements were suggested for inclusion. It was suggested that the option 'to skip the question' should be added in future surveys, as sometimes experts felt they had inadequate information to answer some questions.

Generation of a shortlist of NPEVs

One hundred and six NPEV types were identified from the ICTV website. The *h*-index of all virus types since 2010 ranged from 0 to 53, with a median of 3.5 (IQR 2.0-8.0). NPEVs were shortlisted according to their *h*-index. The ranking of the top 20 NPEVs based on *h*-index is shown in figure 3.5. The shortlisted NPEVs belonged to all 4 species: 9 NPEVs belonged to both EV-A and EV-B species, respectively, and 1 NPEV belonged to EV-C and EV-D species, respectively.

Serotype	H index
EV-A71	53
CV-B3	37
CV-A16	33
EV-D68	26
CV-A6	25
CV-B4	21
EV-A90	21
CV-B5	21
CV-A10	21
CV-B1	20
EV-A92	17
EV-A89	17
CV-A9	16
E-30	16
EV-A91	15
EV-A76	14
E-11	12
E-6	12
E-1	11
CV-A21	8

H-index
50
40
30
20
10

Figure 3.5: Top 20 NPEVs by *h*-index as identified through Web of Science database, 2010-2017. NPEVs are ranked from top to bottom in descending order of *h*-index. Cells are colour-coded by the range of *h*-index.

3.8.4 Second round Delphi survey

The second round Delphi survey sought expert opinion on the revised risk elements, scoring criteria, and the proposed NPEV shortlist.

Survey demographics

Overall, 140 experts were invited to participate in the second round Delphi survey and 39 (28%) full responses were received, 13 of whom also participated in the first round survey. The response rate doubled in the second round. The respondents had experience in multiple disciplines and regions [3]. By region, the respondents had NPEV expertise mainly in Asia (61%, 26/43), followed by Europe (19%, 8/43) and Africa and Middle East (14%, 6/43), compared to 7% (3/43) in America. The NPEV expertise in different WHO regions in the first 2 rounds is shown in figure 3.6. The second round encompassed expertise from a broader geographic area than the first round. The panel consisted of experts with multiple expertise in virology (34%, 26/77), epidemiology (23%, 18/77), clinical medicine (12%, 9/77), immunology (10%, 8/77), vaccinology (8%, 6/77), others (8%, 6/77), bioinformatics (4%, 3/77) and pharmacology (1%, 1/77).

Feedback from second round

Level of agreement with revised risk elements and scoring criteria: Compared to the result in the first round, the levels of agreement with the definitions of risk elements and scoring criteria were increased, with a median increase in agreement of 8.9% (IQR 3.7-12.2) and 14.0% (IQR 12.9-16.2) respectively.

[3]The number of all expertise and regions is used as denominator for the following description, therefore the denominator is greater than the number of respondents.

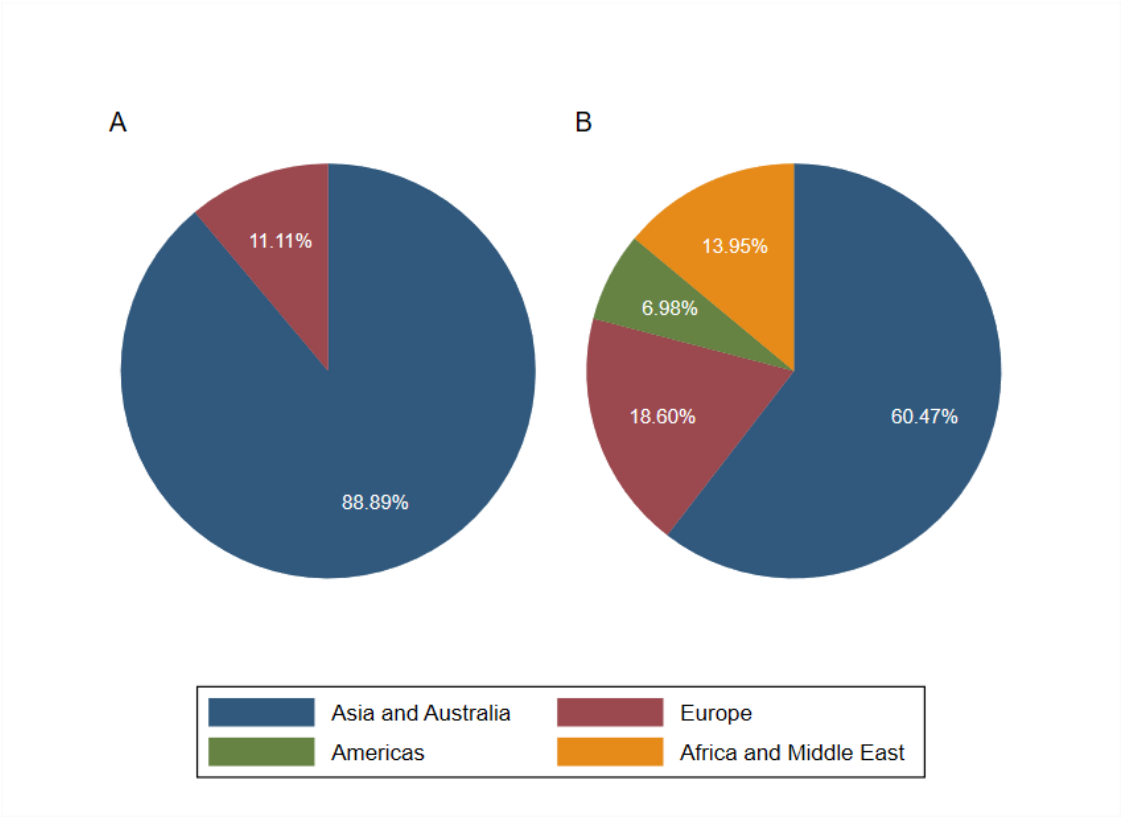


Figure 3.6: NPEV expertise by WHO region in the first two rounds of the Delphi surveys. (A)The proportion of survey respondents' NPEV expertise by region in the first round Delphi survey (n=18). (B) The proportion of survey respondents' NPEV expertise by region in the second round Delphi survey (n=43). Colours indicate WHO regions.

The median agreement level across all risk elements and scoring criteria was high, at 97.4% (IQR 93.6-98.7) and 89.7% (IQR 85.9-93.6) respectively. The levels of agreement ranged from 79.5% for 'incidence and distribution' to 100.0% for 'stage of vaccine development' and 'population susceptibility'. By contrast, the levels of agreement were lower for scoring criteria than that for risk elements, ranging from 64.1% in 'disease severity' to 97.4% in 'stage of vaccine development'.

A median percentage of 2.6% (IQR 1.2-6.4) and 10.3% (IQR 6.4-14.1) of respondents suggested revising the risk elements and scoring criteria, respectively. Risk element modification was recommended most frequently for 'incidence and distribution' (20.5%), 'genetic diversity' (7.7%) and 'disease severity' (5.1%). 35.9% and 15.4% recommended amendment to the scoring criteria for 'disease severity'

and ‘incidence and distribution’, and 12.8% recommended amendment to ‘genetic diversity’. A bar chart of the experts’ opinions on the amendment of the revised risk assessment tool is presented in figure 3.7.

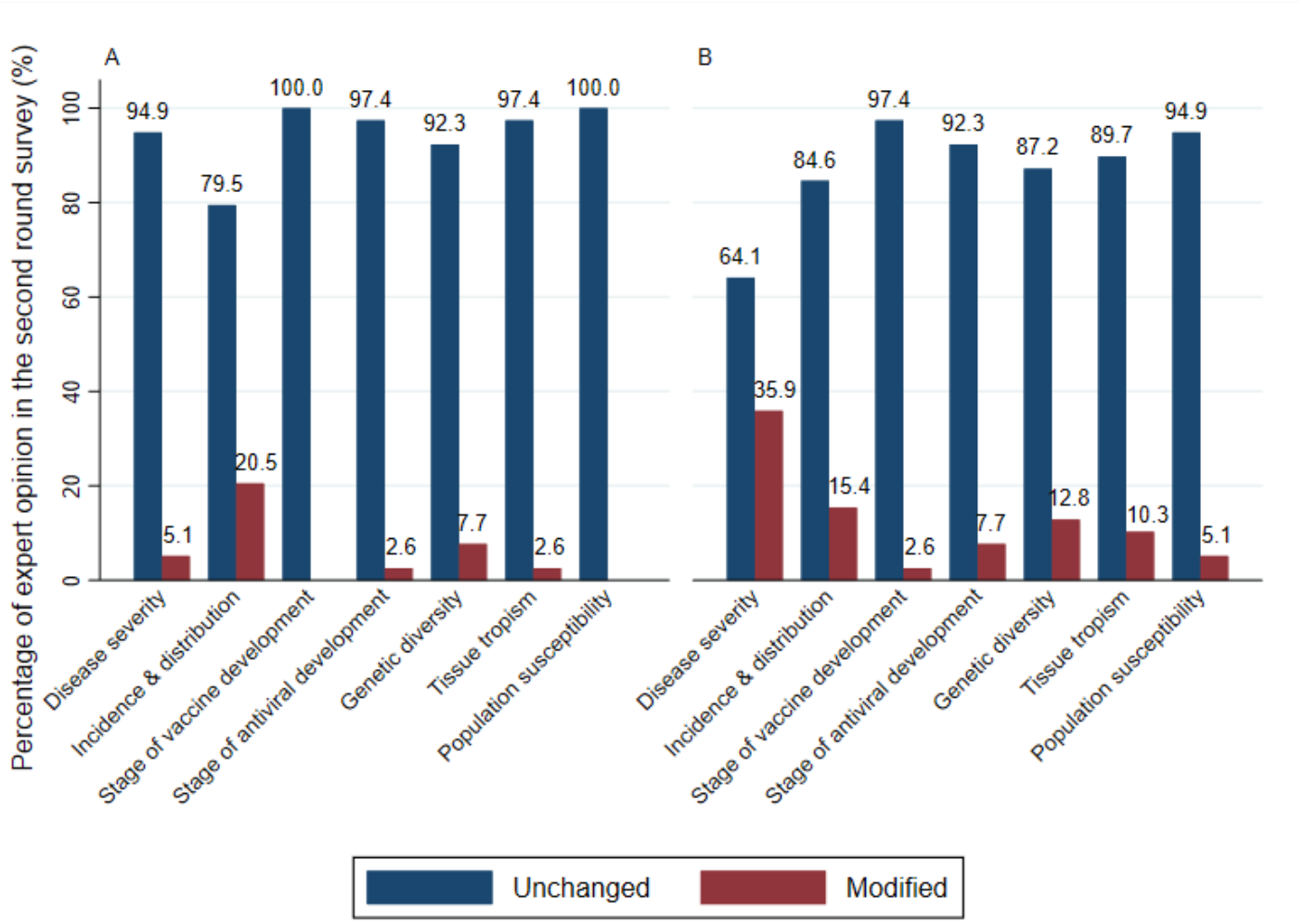


Figure 3.7: Outcome of the second round Delphi survey, expert opinions on tool validation (n=39). (A) Bars represent the percentage of expert opinion on the risk element based on a 2-tiered criteria. (B) Bars represent the percentage of expert opinion on the scoring criteria based on a 2-tiered criteria.

Levels of confidence of experts in reliably selecting scoring criteria There was a high level of data completeness, with a median of completeness percentage at 97.4%, ranging from 94.9% for 'genetic diversity' to 100.0% for 'incidence and distribution'. The mean confidence level was highest in 'stage of vaccine development' (4.34) and 'antiviral' (4.0), followed by 'tissue tropism' (3.95) and 'disease severity' (3.89). The remainder had mean scores of 3.73, 3.62, 3.50 for 'genetic diversity', 'incidence and distribution' and 'population susceptibility', respectively. The completeness and the confidence levels of expert decision on scoring criteria for each risk element is provided in figure 3.8.

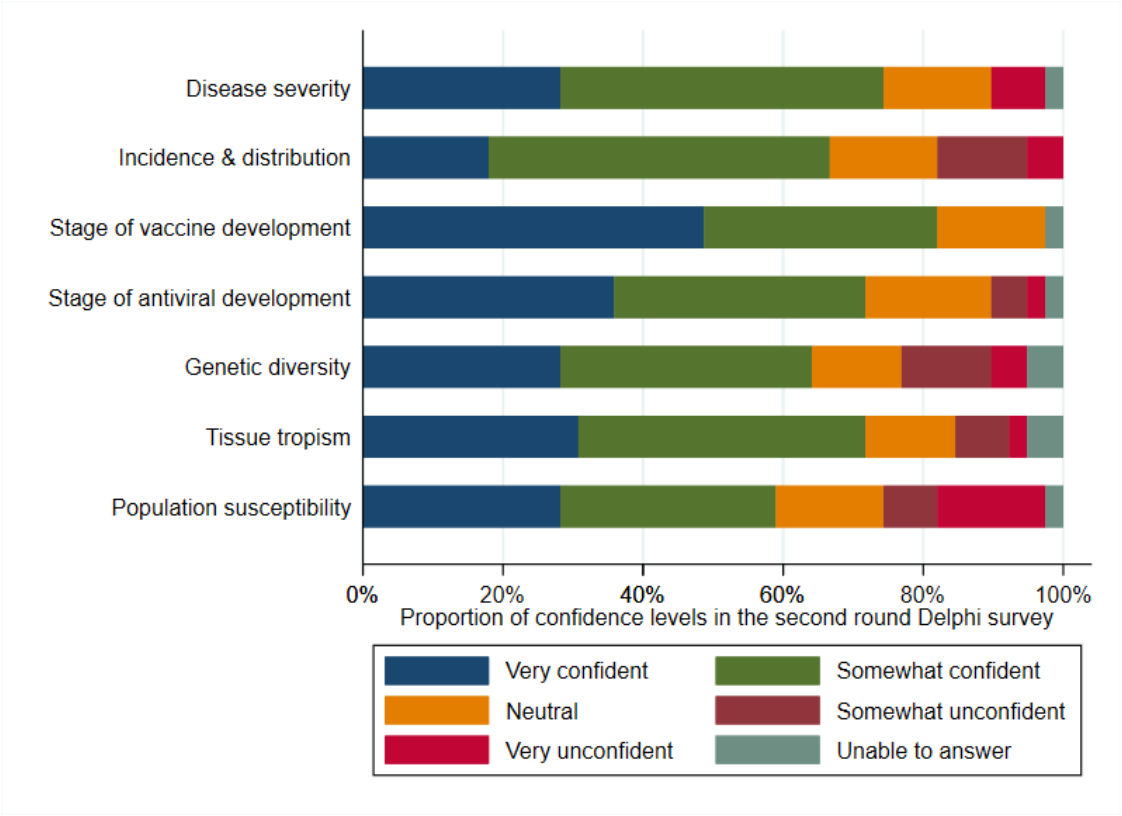


Figure 3.8: Confidence as reported by expert opinions in decision making regarding scoring criteria for each risk element in the second round Delphi survey (n=39). Bars indicate risk elements. Colours represent levels of confidence of experts.

Response to comments on core risk elements and scoring criteria Similar to the first round Delphi survey, amendments in this round included modifying

the definition of the risk element to try to better incorporate the existing evidence and the quality of evidence (1 risk element), and amending the scoring criteria so that they remained mutually exclusive and consistent to the amendment of the risk element.

The main issues raised by experts in the 'disease severity' domain involved the clarification that this was a composite risk element. The definition included both the level of disease severity and the likelihood of developing severe illness. Therefore, we revised the definition by clarifying the element included "the severity of NPEV illnesses and the likelihood of developing severe and moderate illnesses". Several respondents desired the "disease severity" domain to be as quantitative and objective as possible, so we specified categories for the proportions of moderate/severe illness in the scoring criteria.

Regarding the 'incidence and distribution' scoring criteria, we specified that the epidemiological parameters included the incidence of cases, the frequency of outbreaks and the geographical distribution over the past five years. A time frame was added to the scoring criteria.

For the 'stage of antiviral drug' risk element we specified that the antiviral should have specific efficacy against NPEVs rather than efficacy for viral pathogens other than NPEVs. We did not amend the scoring criteria for 'stage of vaccine development' and 'stage of antiviral drug development'.

Response to comments on supplementary risk elements and scoring criteria For 'genetic diversity', we included the diversity of phylogenetically distinct variants in the definition. We did not modify the definitions of the remaining risk elements, but we added 'renal tissue' to the scoring criteria for 'tissue tropism'. We modified the 'at-risk population' to 'children under 15 years of

age' in the 'population susceptibility' scoring criteria. The finalised risk assessment tool is provided in table 3.5.

Validation of selection of a shortlist of NPEVs The expert panel scored each of the 20 NPEVs identified through the h-index for their inclusion (+1) or exclusion (-1) from the shortlist. A zero could be given if the reviewer was uncertain. The sum of scores ranged from -1 to 39. The median of the total score was 16 (IQR 6.5-19). We selected the 15 NPEVs with the highest scores for the initial risk assessment. A bar chart showing the total score for NPEVs based on the three-tiered scale is shown in figure 3.9.

Table 3.5: Final non-polio enterovirus risk assessment tool. The table summarises the core and supplementary risk elements and the corresponding scoring criteria.

Core risk elements

		Risk score		
Risk element	**Definition of risk element**	**1 (Low)**	**2 (Moderate)**	**3 (High)**
Disease severity	The proportion of persons who develop moderate/severe illnesses following infection	Causes almost exclusively mild, self-limiting illnesses. Moderate or severe illness requiring hospitalisation very rarely seen (<1/10,000 clinical cases are moderate or severe)	Moderate or severe illness requiring hospitalisation rarely seen (≥1/10,000 and <1/1,000 clinical cases are moderate or severe)	Moderate or severe illness requiring hospitalisation uncommon but not rare (≥1/1,000 clinical cases are moderate or severe)
Incidence and distribution	A measure of the disease frequency and spatio-temporal trend associated with non-polio enterovirus (NPEV) over the past 5 years	Evidence of no significant change in epidemiology (stable incidence of cases, frequency of outbreaks, and geographic distribution) over the past 5 years	Weak or inconclusive evidence of increasing incidence of cases, frequency of cases, and geographic distribution over the past 5 years	Clear evidence of increasing incidence of cases, frequency of outbreaks, geographic distribution and/or recent emergence as a cause of human disease over the past 5 years

Table 3.5: Final non-polio enterovirus risk assessment tool - *Continued from previous page*

		Risk score		
Risk element	**Definition of risk element**	**1 (Low)**	**2 (Moderate)**	**3 (High)**
Stage of vaccine development	A measure of the development of a candidate vaccine against NPEV and the availability of immunogenicity, safety and efficacy data	Vaccine available or candidate vaccine with demonstrated safety, immunogenicity, and efficacy from at least one phase III trials	Candidate vaccine with safety and immunogenicity data from phase II or observational studies but no phase III trial data	No candidate vaccine or a candidate vaccine with only preclinical or phase I data
Stage of antiviral drug development	A measure of the development of antiviral drugs and compounds with evidence of safety and NPEV efficacy for use in at-risk human populations	Antiviral with demonstrated safety and efficacy from at least one phase III trial	Antiviral with safety and efficacy data from phase II or observational studies but no phase III trial data	No antiviral or a candidate antiviral with only preclinical or phase I data

Supplementary risk elements

		Scoring		
Risk element	**Description**	**-1 (Low)**	**0 (Unknown)**	**+1 (High)**
Genetic diversity	A measure of the diversity of phylogenetically distinct variants and/or the rate of nucleotide substitutions and/or recombination events	Stable genetic diversity of circulating variants (over the past 5 years)	No data or insufficient data	Increasing genetic diversity of circulating variants (over the past 5 years)

Table 3.5: Final non-polio enterovirus risk assessment tool - *Continued from previous page*

		Scoring		
Risk element	**Description**	**-1 (Low)**	**0 (Unknown)**	**+1 (High)**
Tissue tropism	A measure of the type of tissues and cells that support growth of the virus	Data indicate a lack of tropism for human respiratory, cardiac, neurological and/or renal tissue cells	No data or insufficient data	Data indicate tropism for human respiratory, cardiac, neurological, and/or renal tissue cells
Population susceptibility	A measure of the extent of direct or cross-protective immunity to specific NPEV genotype/serotypes in the human populations	Likely to be widespread (>50%) direct or cross-protective immunity in children under 15 years of age	No data or insufficient data	Likely to be widespread (>50%) susceptibility to infection in children under 15 years of age

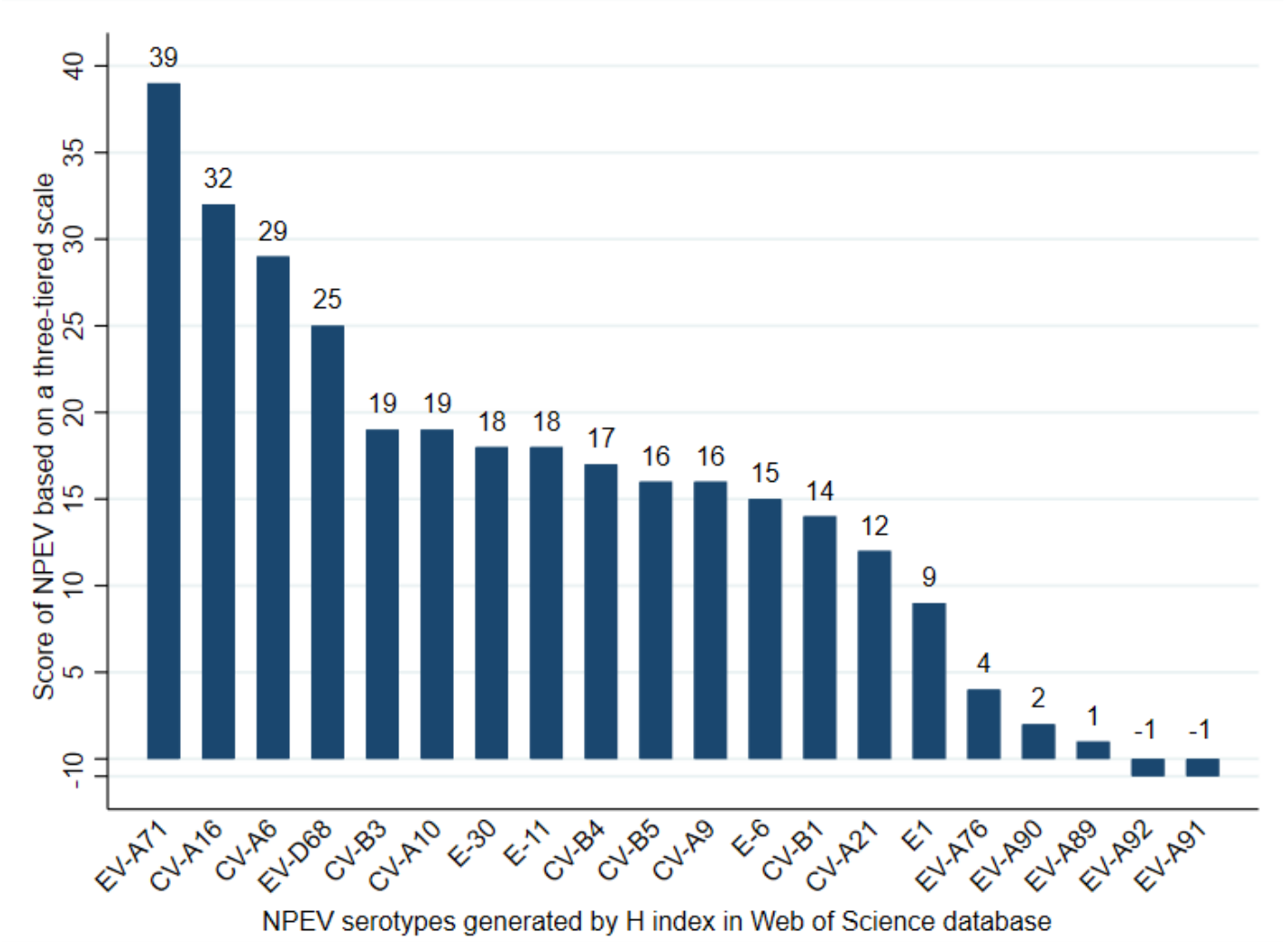


Figure 3.9: Bar chart of validated short-list of NPEVs in the second Delphi survey (n=39). The NPEVs are ranked in order of the total score for shortlisted NPEVs. The height of each bar represents the total score for inclusion, uncertain, and exclusion given by experts.

3.8.5 Final round Delphi survey

The final round Delphi survey sought expert opinion on the levels of public health risk by risk element for each shortlisted NPEV, confidence on their risk assessment decisions, the ranking of risk elements and NPEV research priorities.

Survey demographics

In total, 140 experts were invited to participate in the final survey and 35 (25%) experts provided full responses, 12 of whom had completed the second round survey and 10 of whom had completed all rounds of surveys. Experts had multiple expertise in different regions [4]. By region, the participants had NPEV expertise from Asia (68%, 25/37), followed by Europe (24%, 9/37), Africa (5%, 2/37) and America (3%, 1/37). The NPEV expertise in different regions in the third round Delphi survey is shown in figure 3.10. The expert panel represented a group of multi-disciplinary members with expertise in virology (33%, 24/72), clinical medicine (17%, 12/72), epidemiology (21%, 15/72), immunology (8%, 6/72), vaccinology (8%, 6/72), bioinformatics (6%, 4/72), others (6%) and pharmacology (1%, 1/72).

Completeness of NPEV risk estimates

Across all shortlisted NPEVs, the median number of risk elements completed was 54.3%, with the highest completeness for 'disease severity' (74.3%) and the lowest for 'genetic diversity' (48.6%). The median number of risk elements per NPEV was 57.1%, with the highest completeness for EV-A71 (91.4%) and the lowest for E-1 (48.6%). The data on the completeness of the risk assessment for each NPEV serotype in the final round Delphi survey is shown in figure 3.11.

[4]The number of all expertise and regions is used as denominator for the following description, therefore the denominator is greater than the number of respondents.

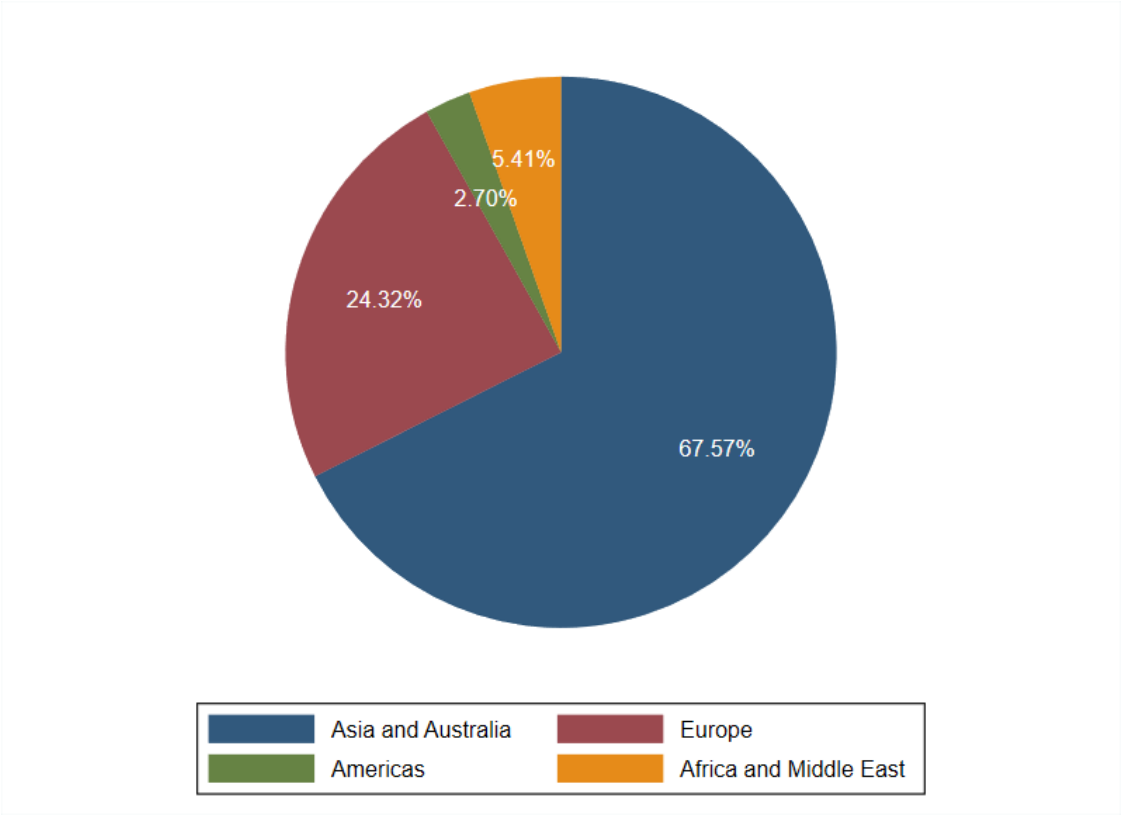


Figure 3.10: NPEV expertise by WHO region in the final round Delphi survey. Pie segments are proportional to the proportions of Respondents. Colours indicate WHO regions.

Risk element / Serotype	Disease severity	Incidence & distribution	Stage of vaccine development	Stage of antiviral development	Genetic diversity	Tissue tropism	Population susceptibility	Average completeness (by serotype)
EV-A71	97.1	97.1	91.4	85.7	80.0	88.6	91.4	**91.4**
CV-A16	97.1	97.1	77.1	77.1	65.7	85.7	85.7	**85.7**
CV-A6	94.3	88.6	71.4	62.9	65.7	77.1	82.9	**77.1**
EV-D68	80.0	82.9	62.9	68.6	60.0	68.6	71.4	**68.6**
CV-A10	85.7	68.6	65.7	54.3	57.1	68.6	65.7	**65.7**
E-30	65.7	62.9	54.3	51.4	48.6	51.4	54.3	**54.3**
CV-B3	74.3	60.0	60.0	57.1	48.6	54.3	54.3	**57.1**
CV-A9	74.3	60.0	57.1	51.4	45.7	51.4	51.4	**51.4**
E-11	62.9	54.3	54.3	48.6	48.6	48.6	51.4	**51.4**
CV-B4	71.4	60.0	60.0	51.4	48.6	57.1	54.3	**57.1**
CV-B5	74.3	62.9	60.0	51.4	51.4	57.1	54.3	**57.1**
E-6	68.6	60.0	54.3	48.6	48.6	51.4	54.3	**54.3**
CV-B1	71.4	62.9	60.0	51.4	48.6	54.3	54.3	**54.3**
CV-A21	54.3	60.0	60.0	51.4	48.6	51.4	51.4	**51.4**
E-1	62.9	54.3	54.3	48.6	45.7	45.7	48.6	**48.6**
Average completeness (by risk element)	**74.3**	**62.9**	**60.0**	**51.4**	**48.6**	**54.3**	**54.3**	**57.1**
Element/NPEV data completeness	**40**	**60**	**80**	**100**				

Figure 3.11: Completeness of NPEV risk score by risk element and NPEV in the third round Delphi survey. Cells are colour-coded by percentage of completeness by risk element for a given NPEV in the risk assessment phase.

Ranking the risk elements by importance

'Disease severity' was ranked as the most important element (weight 0.3704), followed by 'incidence and distribution' (weight 0.2276) and 'population susceptibility' (weight 0.1561), a supplementary element. 'Stage of antiviral development' was ranked as the least important element (weight 0.0204). Respondents were asked to rate importance by assigning a number from 1 to 7, with the most important element being assigned 7, and the least important assigned 1. The overall importance score was estimated by the sum of the assigned value multiplied by the proportion of respondents.The weight of risk elements was calculated according to formula 4.1. The overall importance score ranged from 6.23 to 3.00, with a median of 3.43 (IQR 3.06-4.61). The weight of risk elements ranged from 0.0204 to 0.3704. The rank and corresponding weight of risk elements is shown in table 3.6.

Table 3.6: Importance, rank and weight of risk elements. Risk elements and their respective importance score, rank and weight for calculating overall risk scores for NPEVs.

Risk element	**Importance score**	**Rank**	**Weight**
Disease severity	6.23	1	0.3704
Incidence and distribution	5.57	2	0.2276
Stage of vaccine development	3.43	4	0.1085
Stage of antiviral development	3.00	7	0.0204
Genetic diversity	3.06	5	0.0585
Tissue tropism	3.06	5	0.0585
Population susceptibility	3.66	3	0.1561

The importance scores were estimated by the sum of the rank multiplied by the proportion of respondents who chose the rank in each risk element. The weight of NPEVs was calculated according to formulae 4.1.

Internal consistency of risk assessment tool

A majority of NPEVs (80%, 12/15) had an acceptable Cronbach's α estimate of around and above 0.6. Internal consistency of the risk assessment tool was confirmed for with Cronbach's α being 0.76 for CV-A9, 0.72 for CV-A6, 0.71 for CV-A16, 0.65 for EV-A71, and 0.63 for EV-D68. In contrast, CV-B3, CV-B4 and CV-B5 had an α coefficient lower than 0.6. All the alpha estimates for NPEVs are shown in table 3.7.

Table 3.7: Internal consistency of the risk assessment tool for 15 NPEVs. The scores for all risk elements were used to assess the reliability of an overall risk score by NPEV.

NPEV (sero)type	Cronbach's α coefficient
EV-A71	0.65
CV-A16	0.71
CV-A6	0.72
EV-D68	0.63
CV-A10	0.71
E-30	0.63
CV-B3	0.43
CV-A9	0.76
E-11	0.64
CV-B4	0.54
CV-B5	0.54
E-6	0.60
CV-B1	0.61
CV-A21	0.69
E-1	0.64

Risk score for the shortlisted NPEVs

The overall unweighted risk score for a NPEV was calculated by summing the mean risk score for each risk element according to the formula 4.2. Mean risk score for risk element was adopted because the median risk score yielded identical risk scores for some NPEVs and was not suited for ranking. The overall unweighted risk score for the 15 shortlisted NPEVs ranged from 7.3086 to 11.6992, with a median of 8.8233

(IQR 8.5166-9.3493). A stacked bar chart showing the rank and the composition of the unweighted risk score by NPEV is provided in figure 3.12.

We compared the overall unweighted risk scores calculated by the mean with the overall unweighted risk scores calculated by the median risk scores. The rank for NPEVs remained robust, except for E-6, CV-A10, CV-A16, CV-B1, and E-11 (which had slight changes in ranks). A table summarising the overall unweighted and weighted risk score calculated by mean and median risk scores for the NPEV shortlist is shown in table 3.8.

The overall weighted risk scores for a NPEV were calculated by summing the mean risk score for each risk element multiplied by the weight for that risk element according to the formula 4.3. The overall weighted risk score for the 15 shortlisted NPEVs ranged from 1.0602 to 1.9543, with a median of 1.4349 (IQR 1.3575-1.4962). A stacked bar chart showing the rank and the composition of the weighted risk score is provided in figure 3.13.

After weighting the risk elements, changes in rank were observed for 73.3% of NPEVs. The changes of overall risk scores of the NPEVs before and after weighting the risk elements are shown in figure 3.14.

We compared the overall weighted risk scores calculated using the mean and median risk scores. Similar changes were observed in five NPEV types as in the unweighted risk score comparison. Additionally, EV-A68 ranked the first and EV-A71 the second when median risk score were used, and this was consistent with ranking using overall unweighted risk scores.

Table 3.8: Overall unweighted and weighted risk score for 15 NPEVs. NPEV serotype and their overall risk scores derived in the initial risk assessment.

NPEV (sero)type	Overall unweighted risk score(mean)	Overall unweighted risk score (median)	Overall weighted risk score (mean)	Overall weighted risk score (median)
EV-A71	11.0160	13.0000	1.9543	2.2367
CV-A16	8.6911	10.0000	1.4350	1.5327
CV-A6	9.4049	11.0000	1.5054	1.6412
EV-D68	11.6992	13.5000	1.9510	2.2874
CV-A10	8.4269	10.0000	1.3471	1.5827
E-30	9.3384	11.0000	1.5651	1.6412
CV-B3	9.1774	11.0000	1.4539	1.6412
CV-A9	8.2455	9.0000	1.2898	1.3551
E-11	8.6062	9.00000	1.3742	1.3551
CV-B4	9.1093	11.0000	1.4761	1.6412
CV-B5	9.3602	11.0000	1.4869	1.6412
E-6	8.6854	10.5000	1.3679	1.6119
CV-B1	8.8233	10.0000	1.3705	1.4136
CV-A21	7.3086	8.0000	1.0602	0.9847
E-1	8.0795	8.0000	1.2214	0.9847

The overall risk score for NPEVs was calculated according to formula 4.2 and 4.3.

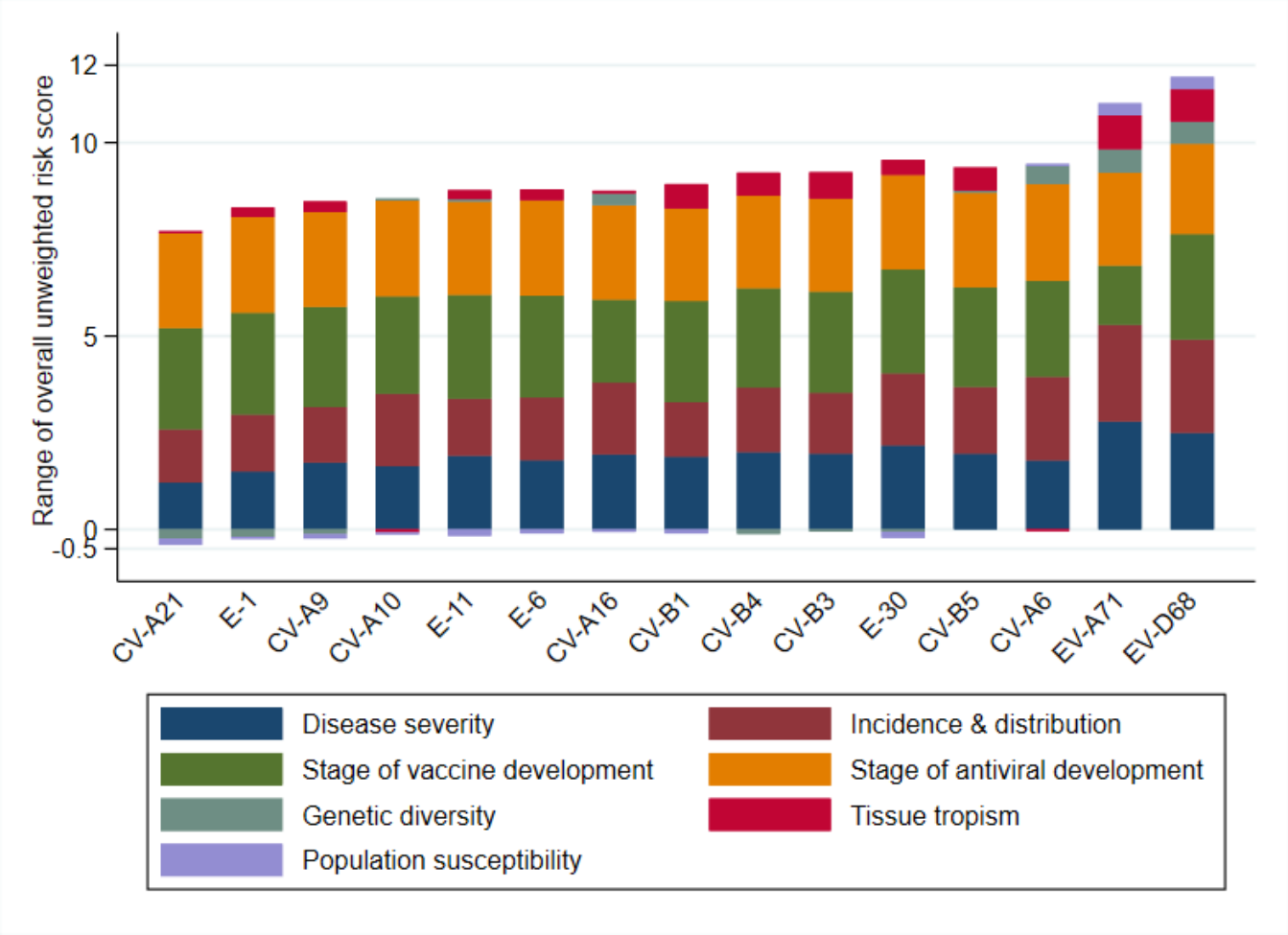


Figure 3.12: Diverging stacked bar chart of overall unweighted risk score by NPEV. The unweighted overall risk score for NPEVs was calculated by the sum of mean risk score for each risk element. NPEVs are ordered by overall unweighted scores. The size of the coloured portion in each bar represents the mean risk attributable to the overall risk score. Colours indicate risk elements. The risk elements are aggregated. The height of each bar shows overall unweighted risk score for NPEVs.

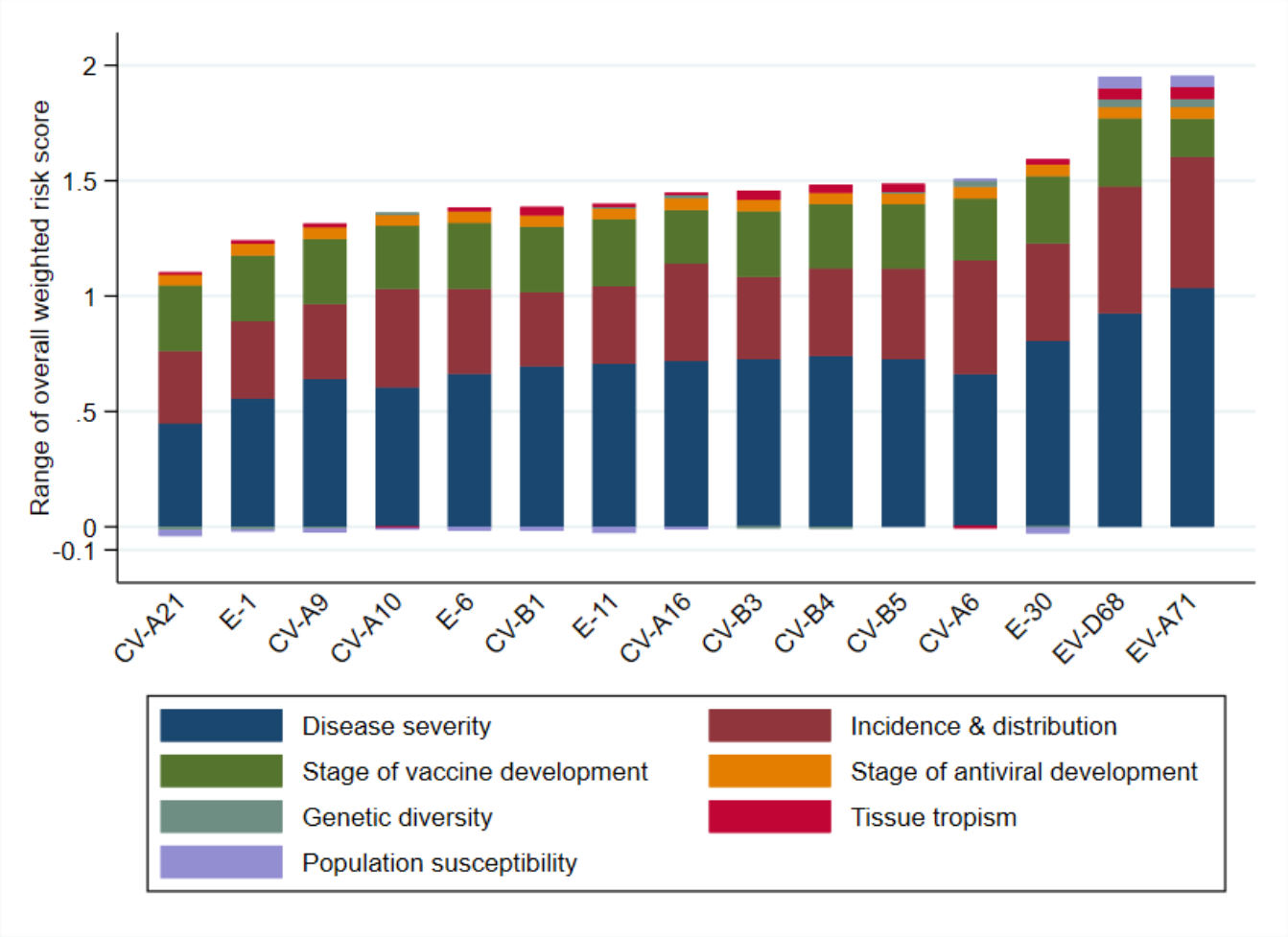


Figure 3.13: Diverging stacked bar chart of overall weighted risk score by NPEV. The overall weighted risk score for NPEVs was calculated by the sum of mean risk score multiplied by the weight of each risk element. NPEVs are ordered by overall weighted score. The size of the coloured portion in each bar represents the weighted mean risk attributable to the overall risk score. Colours indicate risk elements. The risk elements are aggregated. The height of each bar shows overall weighted risk score for NPEVs.

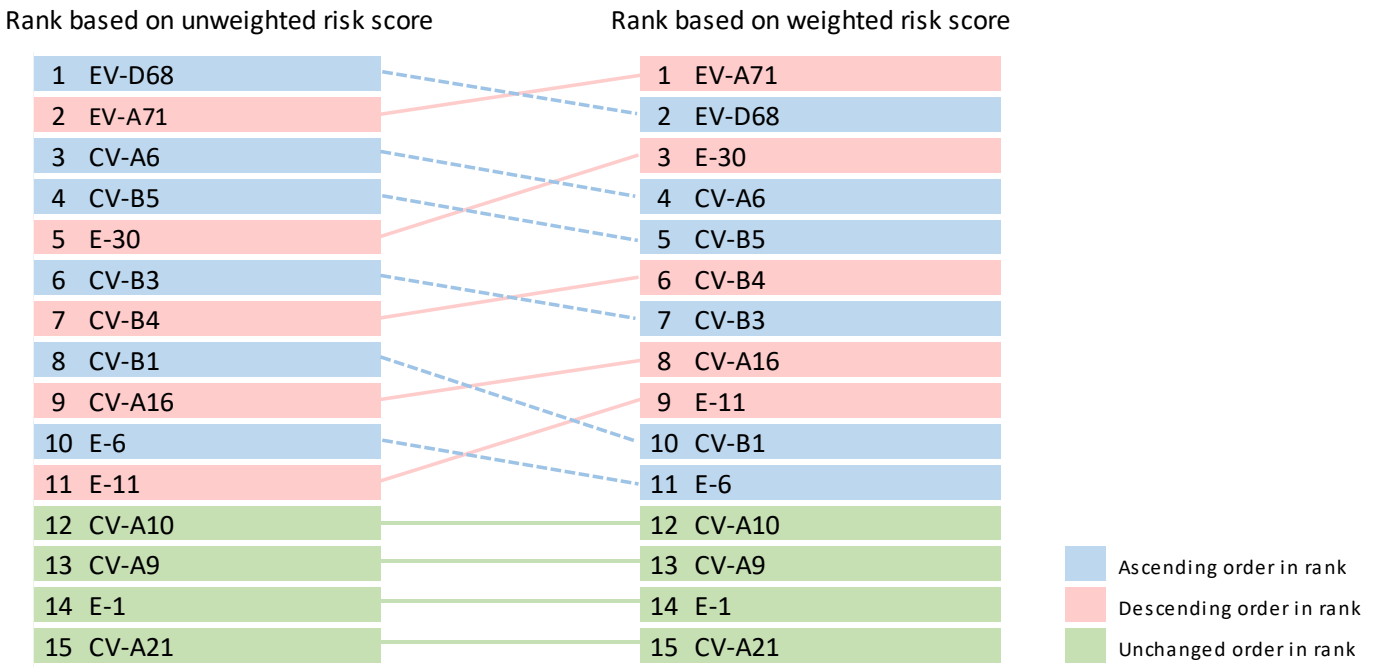


Figure 3.14: Comparison of the top 15 NPEV ranks using unweighted and weighted overall risk scores. NPEVs are ranked from top to bottom in order of the overall risk scores. Solid lines indicate NPEVs that have moved up in rank or stayed the same. Dotted lines indicate NPEVs that have moved down in rank. Colours indicate the trends of rank change.

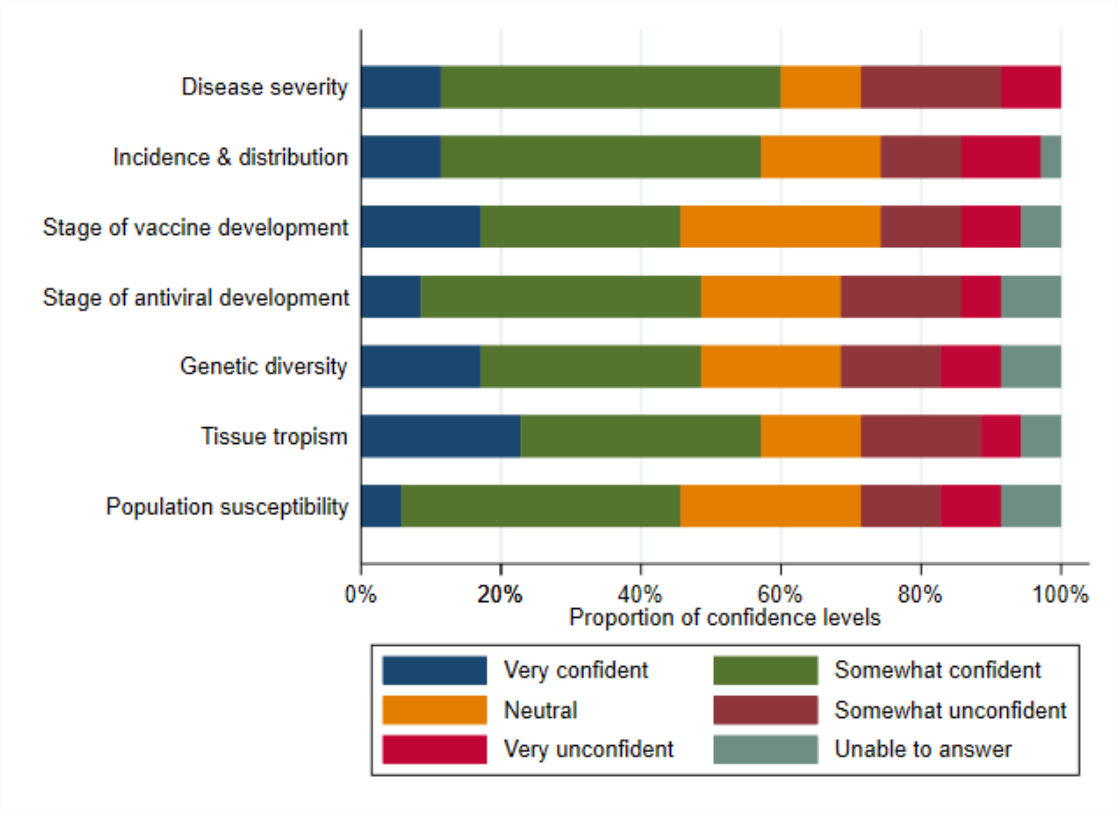


Figure 3.15: Confidence levels of expert opinion by risk element in the third round Delphi survey (n=35). The proportion of confidence levels by risk element in the risk assessment phase. Bars indicate risk elements. Colours represent the confidence levels of experts.

Confidence levels of risk assessment

In the risk assessment, data completeness for the confidence levels ranged from 91.4% for "genetic diversity" and "stage of antiviral development" to 100.0% for "incidence and distribution". The mean confidence level was highest for "tissue tropism" (3.55), followed by "genetic diversity" (3.38), "stage of vaccine development" (3.36), "incidence & distribution" (3.35), "disease severity" (3.34), "stage of antiviral development" (3.31), and "population susceptibility" (3.25). The completeness and the confidence levels of the respondents in the risk elements and scoring criteria are provided in figure 3.15.

Knowledge and capabilities gap analysis and priorities

This qualitative analysis incorporates responses to the open-ended question 'What research would be needed to increase your level of confidence in the assignment of risk?', which was included in both the second and the final round of the Delphi surveys. 31 out of 39 and 32 out of 35 participants in rounds two and three respectively responded to this question, providing 293 comments in total 4055 words of free text. Key themes were identified as described in section 3.7.5.

It was generally acknowledged by the respondents that there are substantial gaps in diagnostics, aetiology, epidemiology and clinical research in spite of the considerable disease burden of NPEV infections. Key data gaps and associated research priorities that would strengthen research and development preparedness were highlighted. A table summarising the key research gaps and future research priorities is shown in table 3.9. Four key themes emerged, these were:

Limitations in diagnostic preparedness The respondents highlighted that a key reason for the scarcity of the global data about the burden of NPEV infections is the small number of cost-effective, sensitive assays that could be used in routine diagnostic settings. In NPEV endemic countries, healthcare facilities will have some capacity to screen for the most-commonly reported NPEV serotypes, such as EV-A71 and CV-A16, whereas other serotypes may not be routinely tested. Cell culture and virus isolation still remain the reference standard for laboratory diagnosis, but the time and resource-consuming nature of this works renders it infeasible for routine diagnostics and for most laboratories. A variety of diagnostic methods exist for detecting viral RNA. These include nested PCR, real-time PCR, reverse transcription loop-mediated isothermal amplification (RT-LAMP) and other approaches. However, the high cost and the resource and skills-intensive nature of these methods limit their widespread use in many daily diagnostic settings. Sensitive diagnostic assays across NPEV serotypes and lineages that could be commercialised

are needed but are yet to be developed. New diagnostic assays should be validated and standardised before being endorsed, produced and used.

Limitations in epidemiological preparedness: Another identified target for filling the knowledge gaps is to improve surveillance and response capacity. Experts unanimously agreed that establishing structured, comprehensive NPEV surveillance systems that are geographically and demographically representative is of public health value. Currently, there is no gold-standard clinical or laboratory surveillance for NPEVs, with an absence of an agreed case definition for severe NPEV illnesses. In primary healthcare settings, laboratory diagnosis still relies on centralised laboratories, which does not yield timely feedback for case management. Improved surveillance is needed to establish a baseline of disease burden that can be used to inform policies, such as immunisation. Most NPEV-endemic countries rely on syndromic surveillance, supplemented by environmental surveillance. Groups at highest risk of NPEV-associated diseases include infants, young children and immunocompromised individuals. Target disease syndromes vary across countries, including aseptic meningitis, acute encephalitis, acute flaccid paralysis, acute respiratory infection, and other acute febrile illnesses. Enhanced hospital- and community-based surveillance linking clinical data with laboratory diagnosis is suggested, not only to define the at-risk populations and regions, but also to characterise the predominant NPEV serotypes and to estimate disease incidence and burden.

Insufficient research and development on medical countermeasures: The experts noted that the medical countermeasure development pipeline for NPEVs is very weak, with only one monovalent vaccine licensed, and this only in China. Ideally, vaccines that allow rapid introduction and deployment should be high-efficacy, heat-stable, multi-antigen, low cost and user-friendly. The aim of research related to vaccine development is to mitigate the burden of epidemics, to prevent severe diseases in infants and young children less than 5 years of age, and to

reduce morbidity in young children. The choice of the most appropriate NPEV strains should focus on antigenically-distinct NPEVs that cause seasonal epidemics and disease burden. A prioritised candidate should be highly immunogenic and well-tolerated. Other consideration of choice include the thermostability of vaccine candidates, because of the limited cold chain and storage capacities in low-income countries. Preclinical evaluation should focus of prioritising vaccine candidates for clinical evaluation, where as clinical evaluation should focus on optimising dose, schedule and timing of vaccination.

With respect to antiviral therapies, not all antiviral targets are subject to the same mutational pressure, with some highly conserved and others highly variable. Therefore, in terms of research and development of antiviral drugs, it is to imperative develop direct-acting antiviral against a broad spectrum of NPEV with long-lasting effectiveness. Therefore, antivirals should ideally target a well-conserved genomic region.

Genomic characterisation and surveillance Viral genes affect the synthesis and packaging of the viral genome and factors that subvert the host immune response. However, it was generally agreed that there are many NPEV populations for which there is little understanding and large geographic regions with limited surveillance. Progress in the development of NPEV medical countermeasures relies on advances in systematic NPEV surveillance and characterisation.

Not all genes of NPEVs are subject to the same evolutionary forces (mutations and recombinations), therefore, assessing contemporaneous genomic diversity is crucial to the choice of specific gene segments for further genetic characterisation and ongoing surveillance. Such evidence will aid efforts to estimate the naturally occurring mutation and recombination rates in NPEVs. For antiviral drug development, this evidence will allow for the choice of specific viral proteins (in highly conserved genomic region) as new antiviral drug targets and assessment of

antiviral drug susceptibility. For vaccine development, this data in conjunction with antigenic characterisation will allow the selection of suitable vaccine viruses by assessing the genetic similarity between the predicted circulating NPEVs and the vaccine strains, and evaluating vaccine-induced immune response and the subsequent immune escape as the viruses evolve.

Coupling the profiling of NPEV genetic profiles with conventional clinical and epidemiological data will provide insights into specific mutations that may be associated with any observed increase in virulence. In support of accelerating antiviral and vaccine development, large-scale genomic surveillance that characterises the natural NPEV genetic diversity is warranted. A possible way forward is to start with a moderate number of surveillance sites and subsequently reduce the number if a similar pattern of genetic diversity is observed in neighbouring regions or populations. Maintaining such surveillance could be performed in a minimum number of key sentinel sites.

Implementation and funding for R&D Aside from technical challenges aforementioned, considerable operational, economic, organisational improvements have been proposed to improve NPEV epidemic preparedness. Most focus on systems-level change, such as supranational collaboration with a worldwide surveillance network or substantial research and innovation funding support. There is also a call for a global data sharing platform with enhanced molecular diagnostic and surveillance capacity. It is important that incentive mechanisms should be effective so that collaboration and financing can be directed toward the most likely threats.

Table 3.9: Research gaps and priorities suggested by expert panel.

	Research gaps	Future research priorities
	Diagnostic preparedness	
1	Few cost-effective and sensitive assays for circulating NPEVs that are more commonly reported	Validation of the clinical performance of available diagnostic assays
2	The lack of rapid tests to diagnose newer NPEVs in outbreak and epidemiological settings (Laboratory capacity to diagnose newly identified NPEVs)	Optimisation and field assessment of in-house diagnostic assays or those in the development pipeline
	Surveillance preparedness	
3	Lack of well-defined NPEV profiles in endemic settings	Continuous enhanced sentinel surveillance to obtain more accurate incidence rates and trends
4	Underestimated number of cases because mild cases who do not present to the hospital	Hospital and community-based surveillance to estimate the disease and economic burden of NPEV illness and infections
5	Undetected asymptomatic infections using the existing surveillance system	Prospective (birth) cohort study to investigate sero-conversion at different age groups
	Emergency preparedness	
6	Lack of deployable vaccines for reactive use in outbreak settings	Structured review for landscape of the target vaccine candidate profiles for clinical-stage assessment Preclinical evaluation of vaccine candidates
7	Vaccines for prophylactic use in groups with higher risk of diseases	Multi-centre clinical trials in prevention of severe complications
8	The lack of therapeutics for first-line treatment	Systematic review of therapy candidates with highest pre-clinical evidence
	Genetic basis for R&D preparedness	
9	Limited understanding of viral determinants as therapeutic and diagnostic targets	Continuous whole genome analysis of different NPEV strains

3.9 Discussion

This is the first study to develop a NPEV-specific risk assessment tool that characterised and prioritised the public health risk of NPEVs using a systematic and transparent methodology. It attempted to support a timely and updatable assessment of the public health risk of NPEVs. It also identified knowledge gaps and R&D priorities so that attention and resources can be dedicated to address unmet needs.

3.9.1 Findings from the literature review

Selection of the methodologies

The literature review allowed us to review the range of methods for risk prioritisation, and to select appropriate methods and attributes associated with the expected outcome during the planning process. Both quantitative and qualitative methods were used for infectious disease pathogens prioritisation. As such, the process of risk prioritisations varied by the prioritisation targets and the methods adopted. Direct methods of data collection or risk prioritisation were considered in the first place. The use of quantitative methods, such as H-index , is easy and quick to obtain for ranking a large volume of pathogens, although the outcome can be limited as it can be subject to time-lag bias and does not take account of the quality of evidence [191]. Therefore, H-index was used for determining the shortlist of NPEVs only. Other direct data collection methods that were commonly used included survey, teleconference, face-to-face meeting, and Delphi technique wherein an effective tool was needed for better communication [142, 163–165, 167, 169–173]. Of these, Delphi technique was selected for data collection for the reasons as follows. First, it is more suited for resolving uncertainty and reaching consensus, Second, the validity and reliability should be a key component of the risk prioritisation [134]. The indicates

the validity of other risk prioritisations remained unclear. As the method required the involvement of a multi-disciplinary panel, better communication should be guaranteed to address inter-rater interpretation and personal interest [191].

We sought to mitigate potential biases. Qualitative risk prioritisation tools appeared more open to interpretation. MCDA, qualitative algorithms, and semi-quantitative approach were the most commonly used qualitative prioritisation methods [163, 165]. In recognition of the fact that complete data and a benchmark for comparing the risk of NPEVs was unavailable, the selection of MCDA for risk prioritisation was justified as follows. First, it can incorporate both expert opinion and empirical evidence and it is easier to interpret than quantitative values and can avoid choices that were too subjective or unreliable [166, 191]. Second, it was characterised by weighting the relative importance of each risk element, which could optimise the usefulness and reflect the importance of the data in each risk element [156]. In addition, it was also endorsed by WHO and the US CDC to assess and prioritise key pathogens that require urgent R&D preparedness [89, 90, 140, 142].

Validity and reliability should be key components of risk prioritisation tools. However, it was noted that only five of the included risk prioritisation exercises employed a validation method, which indicates the validity of other risk prioritisation tools is uncertain [139, 142, 163, 165, 172]. Quality appraisal of the included studies was not conducted as this has been done previously and highlighted the need for a clear and transparent risk assessment tool, which is what we have tried to achieve in this work [191].

Development of the risk assessment tool

The proposed risk elements were selected specific to the context of NPEVs, with reference to the results of the literature review. A wide range of risk elements/criteria have been used in previous risk prioritisation exercises. The inclusion criteria for risk

prioritisation have varied according to their prioritisation purposes and the outcome of interest. However, the most frequently used risk elements (i.e., disease severity, prevention and treatment measures) were selected because they had direct evidence on the pathogen or disease impact and the availability of medical countermeasures. Risk elements that reflected the risk phenotypes, such as genomic variation and transmission in animal models, were also included to improve the risk prioritisation. In this work, we included seven risk elements with varying amount of data. The rationale were given in detail in section 3.8.2.

Most studies encountered the same problem – a lack of reliable data. Concerns arose especially when these data were used to score the criteria for each pathogen [164, 166–168, 172]. To deal with this data gap, we incorporated a predefined option for lack of evidence in supplementary risk elements where data was sparse. In order to close the data gaps, experts were also asked to identify the knowledge gaps and research priorities in the Delphi surveys.

3.9.2 Findings from the Delphi surveys

Risk score and ranking of NPEVs In the risk assessment, expert consensus estimates of relative pandemic risk for a short-list of 15 NPEVs were generated based on seven different risk element and scoring criteria. A weighting method was applied to adjust the results, yielding an overall unweighted and weighted risk score for each NPEV. It was estimated that EV-A71, EV-D68, E-30, CV-A6 and CV-B5 posed the highest public health risk after weighting all risk elements, with overall weighted risk scores ranging from 1.4869 to 1.9543. The overall risk scores were composed of average scores for each risk element. This was useful for determining why NPEVs were ranked in a particular order and what research should be prioritised and performed in response to their emergence.

The NPEVs that pose the greatest threat to public health were identified largely as a result of disease severity and/or increasing trend of incidence and distribution. Despite a vaccine licensed for use in China, EV-A71 was still scored highly for global pandemic potential, with a highest unweighted score of 2.79 and 2.50 for "disease severity" and "incidence and distribution", respectively. This is not only because of the neurovirulence of EV-A71, but also because of the endemicity in most Southeast Asian countries and disease burden over the past decade [46, 105]. In contrast, there is no available vaccine and treatment for other NPEVs, which was indicated by a high average unweighted score for "stage of vaccine development" and "stage of antiviral development" (2.51 and 2.43) for these NPEVs. EV-D68 was also scored highly, with an unweighted score of 2.73 and 2.50 in "stage of vaccine development" and "disease severity". Since 2014, EV-D68 has been increasingly detected during outbreaks of severe respiratory disease and clusters of cases with polio-like illness and meningo-encephalitis [52, 192]. E-30 was another NPEV associated with meningitis syndrome and meningoencephalitis but most frequently reported in Europe [193]. CV-B5 was neurotropic and cardiovirulent NPEV co-circulating in HFMD outbreaks and associated with acute myocarditis and dilated cardiomyopathy [194]. As opposed to the NPEVs aforementioned, CV-A6 was more related to mild HFMD, but it was scored relatively highly due to its increasing extension and intensity of HFMD epidemics among children in Asia and the association with sporadic HFMD in adult populations [195–197].

The ranking of the top 11 NPEVs remained robust, although the risk scores of these NPEVs fluctuated slightly before and after weighting all risk elements. The most notable difference was the increase in rank of E-30 and E-11 and the decrease in rank of CV-B1. This was associated with lower proportions of severe cases in populations, lower incidence rates or narrower geographic distribution.

Reliability and validity of measurement The quality of the risk assessment tool is determined by the reliability and (internal) validity of the risk assessment

tool. Key quality issues are whether the representativeness of the risk elements and the corresponding definitions and clarifications could accurately measure the public health risk of each NPEV, and whether the tool can yield consistent results.

In terms of reliability, we assessed the internal reliability of the first two rounds of surveys. The Cronbach's α coefficient were 0.68 and 0.72, which were reasonably strong evidence for adequate number of questions in the surveys without risking redundancy. We also measured whether the risk assessment tool yielded consistent outcome among NPEVs. The correlation coefficient were 0.95 and 0.93 by using unweighted and weighted individual risk scores, respectively. Although there was evidence for a high degree of reliability when applying the tool, the risk that this might be due to the data gaps for NPEVs cannot be ruled out.

In terms of validity, it is imperative to ensure the representativeness of the risk elements and definite clarification of scoring criteria, because there is no existing "gold standard" tool for assessing NPEV pandemic potential. Therefore, we measured whether the selected risk elements were representative of the important facets of NPEV pandemic risk. All the suggested risk elements met the predefined threshold to be included and we did not receive comments on any additional risk element, indicating main dimensions related to the NPEV pandemic potential should be included. Additionally, we measured how well the risk elements and scoring criteria were defined to incorporate the existing evidence in the first and second surveys, where the median agreement levels were 88.2% and 97.4% for risk elements, and 76.5% and 89.7% for scoring criteria. This indicated a substantial level of content validity of the tool. As the ERAT was intended for NPEVs based on the risk phenotypes in each element, it should have generalisability to any newly-emerged NPEV as well.

Measuring and addressing uncertainty due to data gaps It was noted that there was a lack of empirical data for each risk element. The data completeness

of risk score by NPEV serotype ranged from 48.6% to 91.4% (with a median of 57.1%), while the completeness by risk element ranged from 48.6% to 74.3% (with a median of 54.3%).

Several areas of uncertainty exist in the risk assessment. There was perceived uncertainty as a result of the lack of understanding of NPEV genomic diversity, pathogenicity, evolution and spread, as well as the implications this may have for diagnosis and treatment. Also, other uncertainty is partly due to the variation in terms of the years of experience, experience and familiarity with the published evidence related to NPEVs. Another reason for the scant evidence of NPEVs is due to the small number of cases and a lower proportion of severe cases. Despite a few NPEVs causing epidemics, most NPEV infections are asymptomatic, and the detection of these NPEVs still relies on the reporting of symptomatic cases when the infections become more frequent and cause more severe clinical syndromes [56].

Measures have been taken to address the uncertainty. First, composite risk elements were defined in the tool, where a lack of evidence was indicated in the definitions of core risk elements. As for supplementary risk elements, a scoring criterion for insufficient data was included for use. Second, the level of uncertainty of the risk scores for NPEVs was quantified by expert by using confidence score/level, the median of which were 3.89 and 3.35 in the second and final round survey, respectively. Third, open-ended questions were used to elicit opinions on the identification of these data gaps.

Strengths

Although MCDA framework and Delphi technique are both subjective process to collect varying opinion, definitions were developed for risk elements and scoring criteria to reduce the risk of interpretation and inter-rater bias. A multi-disciplinary expert panel also mitigated the subjectivity and personal interest. Second, this risk

assessment tool was adapted to incorporate empirical evidence and expert opinion. Third, the tool was proved to yield reliable and valid estimates of pandemic potential for NPEVs. Furthermore, this study provide a systematic, standardised, and reliable approach in support of the risk assessment of NPEVs, thereby increasing the reproducibility in later attempts.

Limitations and caveats

There are several limitations of the study. The first is that the response rate was lower in the first round of survey. But we managed to increase the sample size with representativeness of NPEV experts in all four WHO regions. The response rate increased to 28% and 25% in the second and third rounds of surveys to reduce risk of respondent bias [198]. As the response rate was lower than expected especially in the first round, the risk of non-response bias could not be ruled out [198]. One possible reason why the response rate was low is because the recruitment of expert panel was voluntary [199]. However, small biases are often correctable by weighting procedures and may not be as problematic as they at first seem [200]. Second, a non-probability sampling method was used to include experts. Nevertheless, the results were less influenced by the sample size or the geographic representativeness, for these were partly determined by the aim of the study and outcome of interest [134]. Also, the saturation of outcomes is more important for qualitative data [201]. The third is the initial risk assessment faced a paucity of empirical data on the differences between serotypes. This is why the data gap analysis was conducted, which was expected to facilitate research to generate more precise estimates in continuous assessments. Lastly, regardless of how a full risk assessment is performed, it would be resource-intensive to organise and time lags between invitation and experts' response was expected.

There are also a few caveats in application of the risk assessment tool. First, the ERAT is not intended to estimate the probability of causing a future pandemic

or to predict the emergence of NPEVs. Without a complete understanding of all the mechanism and factors associated with the emergence and spread of NPEVs, such estimation still appears difficult at the moment. Second, the NPEV risk may vary if further assessment is performed at national or regional levels, while in this study we assessed NPEVs at global level.

3.9.3 Further application and improvement

To enhance future NPEV risk assessments, knowledge gaps must be filled and much more detailed epidemiological, virological and immunological data are needed.

Factors influencing data gaps

Weak research capacity and inadequate financial investment are some of the factors that probably contribute to the existing knowledge gaps. In the past there has been weak research capacity in developing countries in southeast Asian, resulting in slow advances in diagnostic assays, therapeutics, and vaccines. This has changed in the last five years in China. To improve R&D preparedness, action at global and national levels is warranted to narrow the knowledge gap and expedite the development of medical countermeasures.

Further application of ERAT

The ERAT has undergone considerable refinement and validation and it should be ready for further application. These application could be considered as an external validation to fine-tune the tool if necessary. Although ERAT could be applied to NPEVs when cases are identified in human populations, more accurate estimate still relies on the accumulation of evidence. When more evidence becomes available about the clinical, epidemiological or virological profiles changes of NPEVs,

repeating the risk assessment based on updated knowledge is worthwhile [142]. Timely application of the ERAT would be of value to support decision-making.

3.10 Conclusion

We developed a standardised and reliable risk assessment tool to evaluate the relative public health risk NPEVs. The initial risk assessment has prioritised a short list of NPEV, for which R&D preparedness should be strengthened.

Academic conferences

1. **Cai E**. The development of a non-polio enterovirus risk assessment tool. Second Hand-Foot-Mouth Disease International Conference, Singapore, Republic of Singapore. July 25, 2016. *Presentation*.

2. **Cai E**. The development of an enterovirus risk assessment tool. Oxford Tropical Network, Oxford, United Kingdom. September 8, 2016. *Presentation*.

4

The clinical epidemiology of children hospitalised with hand-foot-mouth disease in Zhengzhou, China

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4.1 Preface to the chapter

This chapter focuses on the characterisation of paediatric patients hospitalised with a diagnosis of hand-foot-mouth disease (HFMD) and meets objectives 3 of the thesis: to conduct clinical research to describe the epidemiology, clinicopathological features, disease progression, case management and outcome in children with HFMD.

4.2 Author's contribution

I designed the study and developed the case report form (CRF) for data collection; I wrote the study protocol, standard operating procedures and the statistical analysis plans; I designed the Access electronic database for data entry in the field; I was responsible for training and managing the data entry operators; I oversaw the data collection in the first round of data entry. I was assisted by the China CDC and Zhengzhou Children's Hospital in Zhengzhou, China in the process of data collection. I cleaned, reconciled and merged all the individual datasets in the first round. I was assisted by Lyndsey Castle in the process of merging the first round datasets. I validated and managed the first full database for preliminary analyses. I co-supervised the second round of data collection and cross-checked the data with the first-round datasets. I collated, cleaned and reconciled and merged the cross-checked datasets for this chapter; I generated data queries to identify missing variables; I validated and managed the cross-checked full database for analysis; I performed all the statistical analyses and produced all the graphs; I drafted all the text in this chapter.

4.3 Abstract

Background: Annual HFMD epidemics cause significant morbidity in China, particularly in children. There are few large-scale studies describing the diversity of clinical manifestations, clinical progression, and risk factors for severe disease. This study aims to describe the demographic and clinicopathological factors associated with disease severity in a cohort of patients admitted to hospital with HFMD in China. Method: A retrospective cohort study of consecutive paediatric patients hospitalised with a clinical diagnosis of HFDM in Zhengzhou Children's Hospital (ZCH) in China. The epidemiology, clinical features, disease progression, management and outcome of patients were described. Both univariate and multivariable statistical analyses of all demographic, clinical and laboratory variables available at presentation were performed to identify factors predictive of disease severity. Findings: Of 6139 patients admitted to ZCH between Jan 1, 2015 and Dec 31, 2016, 6010 (98%) had known outcomes (survived or died) and 129 (2%) were discharged against medical advice. Fifty seven percent of all cases (3508/6139) were classified as HFMD with mild complications and 8% as having severe complications (486/6139) but the overall case-fatality rate was only 0.05% (3 of 6139 patients). Tachypnoea, tachycardia and hypertension on admission were all associated with the development of severe disease. In multivariate analysis, tachypnoea, tachycardia and hypertension on admission, the detection of EV-A71 and rural rural dwelling were all associated with severe complications. Methylprednisolone and mannitol, were frequently given to patients with mild complications. Severe patients also received frusemide, inotropes and intravenous immunoglobulin. Conclusions: Our findings are broadly in line with previous reports of the clinical epidemiology of HFMD. However, a large proportion of patients were reported to have myoclonus, which led to more than half of the cohort being classified as having complicated HFMD. Many of these patient were given mannitol and corticosteroids. Further research is needed to ascertain if isolated myoclonus is an indicator of CNS involvement in HFMD requiring medical intervention.

4.4 Introduction

Hand-foot-mouth disease (HFMD) is a common febrile illness in children, characterized by a typical skin rash, with or without ulcers. Less frequently, more severe complications can develop, presenting with sometimes subtle neurological signs before succumbing dramatically to acute refractory myocardial dysfunction and fulminant pulmonary oedema [33, 202–204]. These complications can develop very rapidly after the involvement of autonomic nervous system, followed by sympathetic hyper-excitation resulting in generalised vasoconstriction with high systemic vascular resistance, decreased left ventricular output, passive pulmonary loading, thereby leading to neurogenic pulmonary oedema. In such cases, death can occur within minutes. This presents particular challenges in terms of case management [205].

China has high rates of HFMD infection and hospitalisation [29, 206] and has had a national HFMD surveillance system since 2008. As of 31 December 2017, a total of 17,908,343 cases, with at least 3,526 fatal cases were officially reported in China [29, 87]. The rate of hospitalisation for HFMD in China has been estimated at 699 to 747 per 100,000 population [207, 208]. Previous retrospective and prospective clinical cohorts of children hospitalised with HFMD have reported a diverse group of NPEV serotypes circulating in China mainland, constituting approximately 90% of all the cases in West Pacific Asia per year. The reported rate of severe or complicated cases has ranged from 10-30%, although these studies had different target populations and enrolment criteria [86, 209, 210]. The case fatality rate has been more consistent, generally less than 1% but ranging from 0.8-3%.

Factors associated with a severe clinical outcomes have included young age [86, 211], female gender [212], male gender [213], the presence of lethargy [212], the absence of a rash [211, 213, 214] and infection with EV-A71 (as opposed to other serotypes) [86, 209, 212, 214]. Liu et al recently developed a 16 variable tool (named HFMD-RF) to predict which children were at risk of deterioration,

with LDH, CK-MB, blood glucose, CK and vomiting being the strongest predictors [215]. However, this 16 item tool is likely to be difficult to use in practice and has not been independently validated.

The associated of severe disease with poor long-term outcomes, such as neurocognitive impairment, is variable, with some studies reporting high rates of sequelae, [216, 217] whereas others report high rates of full recovery [218].

Supportive care remains the principal management strategy for patients with HFMD. Recommended components of care in China included oral or intravenous fluids, antipyretics, sedatives, corticosteroids, vasoactive medications alongside empirical antimicrobials. Management of ANS dysregulation focuses primarily on hemodynamic abnormalities, particularly hypertension. Milrinone was used to control high blood pressure and to support myocardial function. The use of milrinone also reduced the mortality associated with pulmonary oedema. Intravenous immunoglobulin is widely used for cases of HFMD with neurological complications, and is recommended by WHO and several national guidelines but its safety and efficacy has never been evaluated in a clinical trial [219, 220] .

The largest clinical cohort reported to date, consisting of 7203 children, was assembled by Qiu et al [209], in Hunan province China, but provided little clinical data. In order to better characterise the clinical epidemiology of HFMD in hospitalised children and to provide a more comprehensive description of their management and outcomes, we assembled a large and detailed clinical dataset of all children admitted over a two-year period to a large referral hospital in a Chinese province with high reported incidence of severe and fatal HFMD.

4.5 Aim and objectives

The overarching aim of this work is to characterise paediatric patients diagnosed with HFMD in an epidemic setting. The first objective of this work was to describe the epidemiology, clinical features, course, management and outcome of patients with HFMD. The second objective of the work was to establish predictors associated with disease severity.

4.6 Materials and methods

4.6.1 Study design

Retrospective cohort study

We conducted a retrospective cohort study of children hospitalised with HFMD. We used retrospective chart review (RCR), known as medical chart review. RCR is an important methodology that generates hypotheses or answers research questions using historic (pre-recorded) patient data. Many forms of data are used in such reviews: electronic database, physician and nursing notes, admission and discharge notes, consultations, laboratory and diagnostic testing records, and administrative records, etc [221]. The use of readily accessible data provides a time and resource efficient method to investigate medical conditions with rare occurrence or a long latency between exposure and outcome, to establish causal relationships, and more importantly, to generate hypotheses [221, 222]. With these advantages, this methodology has been widely applied in a range of diseases and settings [179, 223, 224].

The limitations of retrospective chart review include incomplete documentation, missing charts and difficulty in verifying and interpreting information found in

the documents. In view of the retrospective nature, methodological guidance on planning and conducting RCRs was followed to minimise the impact of the recognised limitations and to ensure consistent and integral data collection [221, 222, 225].

Site selection

ZCH is a tertiary public hospital and, with 2,200 beds, is the largest paediatric hospital in Henan Province. It serves the population of Zhengzhou city which, according to the Zhengzhou Bureau of Statistics, has a total population of the study area 9.6 million in 2015 and 9.7 million in 2016, of which 21.2% were children aged 0-14 years. Each year ZCH provides an estimated 1.25 million outpatients consultations and admits approximately 86,000 inpatients. ZCH has a 55-bed infectious disease department and a 20-bed paediatric intensive care unit including a 16-bed high dependency unit beds and an 8-bed infectious disease high dependency unit that accommodates children with clinically suspected HFMD and other infections. Attending patients are referrals from regional primary or secondary healthcare centres and a mix of self-referrals from nearby neighbourhoods and [226].

Participants

In 2015 and 2016, 2,014,999 and 2,468,174 cases of HFMD were reported to the national surveillance system in China [87]. In these years Henan Province reported 86,359 (4.3%) and 128,039 (5.2%) of all cases [227]. In ZCH, 27,591 and 39,904 patients sought health care due to HFMD/ Herpangina (HA) in 2015 and 2016, and made up 31.9% and 31.2% of all notified patients in Henan. Of the ZCH cases, 2,560 (9.3%) and 3,874 (9.7%) were inpatients.

According to the Chinese HFMD clinical guidelines, the presence of at least one of the following characteristics is considered an indication for hospitalisation at a tertiary care facility: age<3 years, disease onset < 3 days, a diagnosis of severe

HFMD, organ involvement or any other unstable conditions requiring emergency care [33]. Criteria for ICU admission include, but is not limited to, the following conditions: patient with severe or potentially life-threatening pulmonary conditions (patients ventilated or potentially needed ventilation), cardiovascular (shock due to any shock, post cardiopulmonary resuscitation, unstable cardiorespiratory status), neurologic (acute and deteriorating consciousness), gastrointestinal diseases, or metabolic conditions.

Between 1 January, 2015 to 31 December, 2016 children admitted to ZCH with a discharge diagnosis of HFMD were identified from the electronic medical systems and screened for eligibility. We used the *International Classification of Diseases, 10th* ICD-10 diagnosis code B08.4 (Hand Foot and Mouth Disease and Herpangina) and the corresponding free text term in Chinese in the discharge diagnoses field to identify patients with HFMD in the hospital electronic medical systems.

Eligibility criteria

Eligibility criteria allow excluding incomplete and inaccurate data to aggregate a relatively homogenous sample that is sufficiently large to represent the population and could be analytically approached [228, 229]. In order to study a range of outcomes including more severe outcomes and to ensure the representativeness of the sample, we included children under 18 years of age consecutively admitted to ZCH between 1st January 2015 and 31th December 2016 with a diagnosis of HFMD or herpangina. We excluded patients providing incomplete data, with underlying comorbidities and patients with sequelae after HFMD. The exclusion criteria were the following: (1) patients discharged within 24 hours; (2) patients with long-term sequelae of prior HFMD disease who were admitted to ZCH for rehabilitation; (3) patients whose primary reason for admission was a condition other than HFMD; (4) patients who had comorbidities on admission whose symptoms and signs overlapped with the complications of HFMD; (5) patients who had an

established diagnosis other than HFMD and neurological complications or patients who had a unclear diagnosis by the time of discharge.

Data collection and processing

A standardised case report form (CRF) was designed to conduct a study characterising the patients and another study assessing the usefulness of the existing HFMD guidelines. It was used to extract information on eligibility, demographics, previous hospitalisation, past medical history, concomitant conditions and medications, presenting symptoms, daily symptoms and signs, laboratory tests, imaging assessments, diagnostic summary and therapeutics.

The CRF included sections corresponding to these contents. Each section had a heading and questions or prompts. Tick boxes, drop-down lists and multiple choices were used to record data with pre-determined choices, boxes were provided to record free-text or numbers where it was necessary. Instructions were given to provide definitions or guide the data entry. Skips and default values were used to simplify the data entry. Validation rules were set up to ensure valid and complete data entry. A database password was used to protect the data. Variable names, definitions, types, values and labels were included in a data dictionary.

Based on the CRF, we built an electronic database in Microsoft Access 16.0; input forms (user interface) were used for data entry and tables for data storage. An eight-digit unique identification number was assigned to each patient, which was also used as index variable to link all tables. Anonymised data from patient admission to discharge were extracted from electronic case notes and entered in the Access database. After data collection was complete, data were transferred using OxFile, the University of Oxford encrypted file exchange service. Individual databases for each table were exported and appended in Microsoft Excel 2016

using Visual Basic for Applications. Further table merging and data cleaning were performed in STATA (version MP 15.0).

Data anonymisation

Efforts were made to protect personal data without affecting double data entry. Data de-identification and anonymization were achieved following the 'Safe Harbour' standards, namely removing eighteen listed types of identifiers according to the guidance [230, 231]. No personal identifiers were actually included in the standardised data collection form or the electronic Access database, or entered in any data subsets by students.

To resolve discrepancies and retrieve missing key variables, a separate file including the unique ID and the medical record number only was stored securely on the hospital intranet. This file could only be accessed by one or two authorised people. The file could not be copied, downloaded or sent through intranet. Another collaborator managed and destructed the file when data merging and cleaning were finalised.

Enterovirus detection and serotype determination

Enterovirus detection and serotype determination Routine practice at ZCH is to collect a stool specimen or rectal swab in patients with suspected NPEV infection to perform enterovirus PCR. The presence of generic (pan-enterovirus) and specific (EV-A71 and CV-A16) viral RNA (see Appendix) is assessed by multiplex real time RT-PCR (Jiangsu Mole, China) using the real time quantitative thermal cycler (TIB-B600, China). The reactions of the real time RT-PCR were performed under the following conditions: an initial cycle at 45 degrees for 15 min, one cycle at 95 degrees for 2 min, followed by 40 cycles of 94 degrees for 10s and of 58 degrees for 40s [232].

Serological tests were performed in serum and CSF by using a commercial EV-A71-, coxsackievirus- and echovirus-IgM-colloidal gold immunochromatographic assay (Beijing Wantai, China) approved by China Food and Drug Administration. The turnaround time was 90 minutes. The diagnostic kit allows for IgM detection within 1 or 2 days after disease onset.

For the purposes of analysis, a laboratory confirmed case was defined as a patient with a clinical diagnosis of HFMD or herpangina and either a positive PCR result for EV-A71 or CV-A16 or pan-enterovirus. A probable case was defined as a clinical diagnosis of HFMD or herpangina and a positive IgM result for either EV-A71, coxsackievirus or echovirus but without PCR evidence for EV-A71 or CV-A16 or pan-enterovirus.

Outcomes

Outcomes included inpatient mortality, ICU admission, and any complications as recorded by the clinicians. Complications listed in the WHO HFMD case classification system [33] were extracted from patient records, these were: aseptic meningitis, brainstem encephalitis, encephalitis, encephalomyelitis, acute flaccid paralysis (AFP), autonomic nervous system (ANS) dysregulation, pulmonary oedema/ haemorrhage and cardiorespiratory failure.

Definition of uncomplicated and complicated HFMD For the purposes of analysis we defined three categories of HFMD: (i) uncomplicated HFMD; (ii) HFMD with mild complications; and (iii) complicated and critically ill HFMD. The signs, symptoms or clinical interventions used to categorise patients are shown in table 1. The presence or absence of each variable was assessed for each day of admission and the patient was placed in the highest category that they achieved during their admission. Sepsis was included as a 'mild-complications' since the term 'sepsis' was

used by clinicians to describe an inflammatory state (the occurrence of at least one of the following criteria: fever or hypothermia, tachycardia, tachypnea, leucocytosis or leukopenia) rather than life threatening organ dysfunction or septic shock.

Table 4.1: Uncomplicated and complicated HFMD definition.

Variables	Uncomplicated HFMD*	HFMD with mild complication*	Complicated and critically ill HFMD*
History of fever OR fever on admission (>38°C)	✓	✓	✓
Skin lesions on palms, soles or buttocks	✓	✓	✓
Oral ulcers	✓	✓	✓
Altered level of consciousness	×	✓	✓
Neck stiffness	×	✓	✓
Photophobia	×	✓	✓
Seizure	×	✓	✓
Myoclonus	×	✓	✓
Tremor	×	✓	✓
Ataxia	×	✓	✓
Decreased muscle tension	×	✓	✓
Limb weakness	×	✓	✓
Unsteady gait	×	✓	✓
Abnormal respiratory pattern	×	✓	✓
Sepsis	×	✓	✓
Aseptic meningitis	×	×	✓
Encephalitis	×	×	✓
Brainstem encephalitis	×	×	✓
Encephalomyelitis	×	×	✓
Acute flaccid paralysis	×	×	✓
ANS dysregulation	×	×	✓
Cardiac arrest	×	×	✓
Myocarditis	×	×	✓
Pulmonary oedema	×	×	✓
Pulmonary haemorrhage	×	×	✓
Severe sepsis	×	×	✓
Multi organ failure (MODS)	×	×	✓
Heart failure	×	×	✓
Respiratory failure	×	×	✓
Circulatory failure	×	×	✓
Shock	×	×	✓
Brain herniation	×	×	✓
Cardiopulmonary resuscitation	×	×	✓
Ventilator use	×	×	✓
ICU admission	×	×	✓
Hospital mortality	×	×	✓
Out-of-hospital mortality	×	×	✓
Cause of death	×	×	✓

* Symptoms defining the case severity is indicated by ticks and green shades in each column. Case severity is defined based on the principle of mutual exclusivity.

Vital signs and laboratory results Vital signs and laboratory results were classified as low, normal, or high based on age-specific normal ranges obtained from the Royal College of Nursing and East Sussex Healthcare reference range handbook [233, 234].

4.6.2 Ethics and data transfer agreement

The study was approved by Oxford Tropical Research Ethics Committee(528-18), the Medical Research Ethics Committee at China CDC and Zhengzhou Children's Hospital. A data transfer agreement has been established between ZCH and the research group in Oxford. Data collection, transmission, storage, security, analysis and destruction have complied with international and local regulatory standards [235–237]. Risk of a privacy breach was eliminated.

4.6.3 Data management

Database controls

Quality assurance procedures were built into the Access database. These procedures included setting the variable format type, variable measurement scales, default values, decimal places, the use of validation rules and texts. Standard Operating Procedures for accessing the hospital electronic system, screening patients, using the Access database, entering and saving data were established.

Piloting, training and monitoring

Medical students were recruited for data entry. Each student received intensive orientation and training before being granted access to the database to pilot the database or start data entry. We asked medical students to pilot the CRF and

the electronic database was adapted correspondingly. This was done in order to make sure all the settings in the electronic database were functioning and captured complete, consistent and valid data with a user-friendly interface [238–240]. Such adaptions included but were not limited to testing the correctness of instructions, restricting data input by adding or refining validation rules, and streamlining the standard operating procedures for data entry. A one-month training and piloting period of the CRF and database took place before the formal start of the data collection. During the data collection, we were physically present and oversaw the data entry throughout the study to make sure the quality assurance procedures had been followed by daily monitoring of data (including the patient screening, data storage and data completeness in key tables, etc). Double data entry was performed by independent medical students in separate files. The first and second batch data were compared. Discrepancies were identified by a validation Access database and resolved by verification against the original case notes at the field site before being transmitted [239, 240].

Data transfer and cleaning

Upon receipt of the verified dataset in Oxford, extensive data checking and cleaning were implemented, starting with checking for missing data, duplicate records, detecting and correcting logic errors or range errors, and ending with validating and reconciling data inconsistencies. More specifically, missing patients and key variables, such as unique ID, age, gender and diagnosis, were first identified. For missing key variables, queries were generated in Microsoft Excel and sent to the field site to request missing variables. The incoming data from the field were updated in the verified dataset. Once data cleaning was complete, the clean dataset was locked and formatted for analyses.

4.6.4 Statistical analysis

Descriptive statistics were tabulated or summarised as frequencies and proportions for categorical variables and were analysed with the chi-square test and Fisher's exact test, as appropriate. For continuous variables, normality was tested with Kolmogorov-Smirnov test. Mean and standard deviations were reported for normally distributed continuous data and were compared with the use of Student's t-test. Skewed distributions were described with medians with interquartile ranges and were compared with the use of the Wilcoxon rank-sum test and Kruskal-Wallis test.

Odds ratio and 95%CIs were calculated by Mantel-Haenszel test. Candidate variables, all assessed within the first 24 hours in the hospital, were selected based on research evidence or clinical experience from published literature. Univariate associations with outcomes were calculated for variables with at least 50% of data available. Multivariate stepwise logistic regression models were built to evaluate the associations between these variables and the outcomes, with forward selection procedures. Likelihood ratio test (LRT) was used to compare a simple model (null hypothesis) with a more complex model (the alternative hypothesis. A P value < 0.10 indicated the inclusion of the tested variable in the LRT. All P value were calculated from two-tailed tests of statistical significance with a type I error of 0.05. Analyses were conducted using STATA (version 15.0).

4.7 Results

4.7.1 HFMD patients

Between January 01, 2015 and December 31, 2016, 6434 relevant records were identified from the electronic medical system: 2560 (40%) paediatric patients were admitted in 2015 and 3874 (60%) in 2016. So in total, 6434 patients were admitted

and after removing duplicate records, 6425 patients were screened for eligibility for the study. Of these 265 and 21 were excluded based on eligibility criteria and data availability respectively. Data was therefore available on 6139 patients for analysis. The dataset for analysis comprised 6139 patients. The process of patient screening and inclusion is shown in figure 1. According to figures from China CDC, Henan had the fifth highest HFMD morbidity during these two years in China, with the third highest number of fatal cases, at 26 for those two years (the second highest number of fatal cases in 2015) [227].

4.7.2 Data completeness

Record completeness for eligibility criteria was 93.1% in the initial dataset but increased to 100% in the final dataset after resolving missing data through data queries to the field site and excluding those whose eligibility data was still missing. Data completeness for key variables is shown in tables 4.2. Data on demographics, comorbidities and on the time interval from illness onset to admission showed completeness of greater than 98% (table 4.3). Completeness of data on signs, symptoms, vital signs, laboratory results and treatment are reported in the corresponding result sections below.

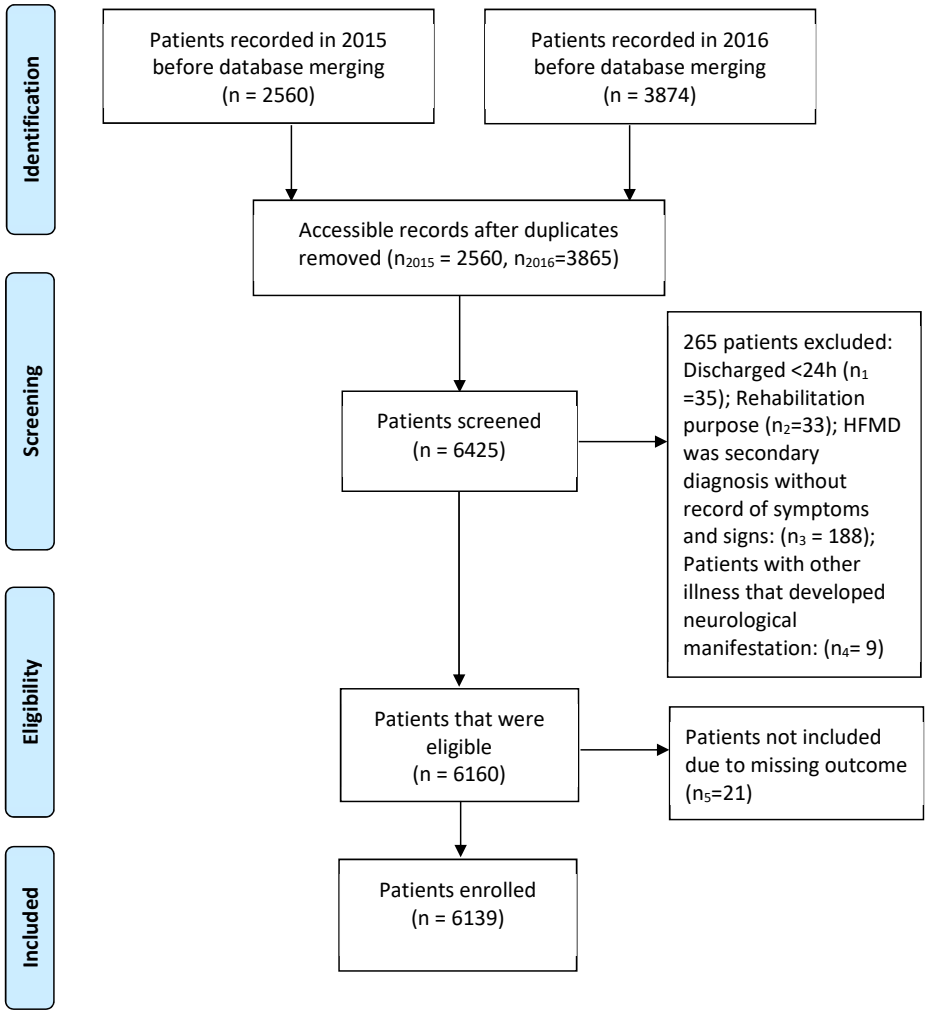


Figure 4.1: Flow chart of patient selection process.

Variables	Completeness-n (%)
Key variables	
Subject ID	6425(100)
Age	6139(100)
Sex	6139(100)
Inclusion criteria	
1. Timeframe from 2015/01/01 to 2016/12/31	6425(100)
2. Patient had a diagnosis of HFMD	6425(100)
3. Patient had a diagnosis of HA	6425(100)
Exclusion criteria	
1. Patient discharged within 24 hours	6425(100)
2. Prevalent patient with long-term sequelae who admitted for rehabilitation treatment	6425(100)
3. Patient who had HFMD/HA as a secondary diagnosis, and symptoms and signs were not recorded in detail	6425(100)
4. Patient who had an established diagnosis other than HFMD and its associated complications or had an unclear diagnosis by the time of discharge.	6425(100)
Outcome*	
HFMD severity grades	6139(100)
Survival at discharge	6010(98)

* The completeness of HFMD associated complications were recorded in daily case notes, and aggregated as summary variables for validation, therefore, the completeness was not assessed.

Table 4.2: Data completeness of key variables, inclusion, exclusion and outcome variables.

Variables	Completeness-n (%)	Variables	Completeness-n (%)
General		Familiar factor	
Subject ID	6139(100)	Mother's age	6080(99.0)
Age	6139(100)	Fathers' age	6080(99.0)
Sex	6139(100)	Maternal, perinatal, infant, and childhood factors	
Ethnicity	6129(99.8)	Parity	6085(99.1)
Area of residence	6119(99.7)	Caesarean delivery	6083(99.1)
Education	6124(99.8)	Preterm birth	6085(99.1)
Comorbidities*		Low birthweight	6010(97.9)
Epilepsy	5829(94.9)	Feeding history	6076(99.0)
Diabetes	5826(94.9)	Immunisation status	5900(96.1)
Asthma	6128(99.8)	Physical factor	
Upper respiratory tract infection	6128(99.8)	Weight	6076(99.0)
Lower respiratory tract infection†	5498(89.6)	Healthcare seeking and services	
Infective diarrhoea	6128(99.8)	Preadmission location§	6139(100)
Other infections	6128(99.8)	Time from disease onset to first health facility	4128(66.7)
Other diseases requiring treatment or surgery	6128(99.8)	Time from disease onset to admission	6056(98.6)

* Some rare medical conditions were recorded as free text in a text box, therefore, does not have a denominator for calculating completeness.
† Composite variable: including bronchitis and pneumonia.
§ Composite variable: Time of admission to health facility 1-3.

Table 4.3: Completeness of demographic, comorbidity and baseline variables.

4.7.3 Residence of study subjects

Complete information about residence area (at county level) was available for 6129 (99.8%) of 6139 patients. Of these, 6058 (99%) were from Henan Province, with 3521 (57%) from Zhengzhou and the remaining 2537 (41%) from 28 neighbouring cities or prefecture-level cities. Only 71(1%) subjects were from other provinces. 2888 (47%) were urban dweller, while 3231 (53%) were rural dweller. The geographic distribution of cases admitted to ZCH during the study period and that of the contemporary cases in China are shown in figure 4.2. The patients in this study comprised around 3% of all reported HFMD cases in Henan Province in 2015 and 2016.

4.7.4 Seasonality of admission

The monthly number of the HFMD admission exhibits an annual peak that occurs in April, although the peak in 2016 lasts a longer period till August. In 2015, the admissions increases drastically around April and peaks at 687 in May, followed by a gradual decrease starts in July, constituting 63% of the annual admissions. The peak in 2016 shows a similar seasonal pattern, with the highest monthly number of admission of 615 that occurs in June. However, the total admission in 2016 increases by 50% as opposed to that in 2015. The temporal patterns of the HFMD admission is shown in figure 4.3.

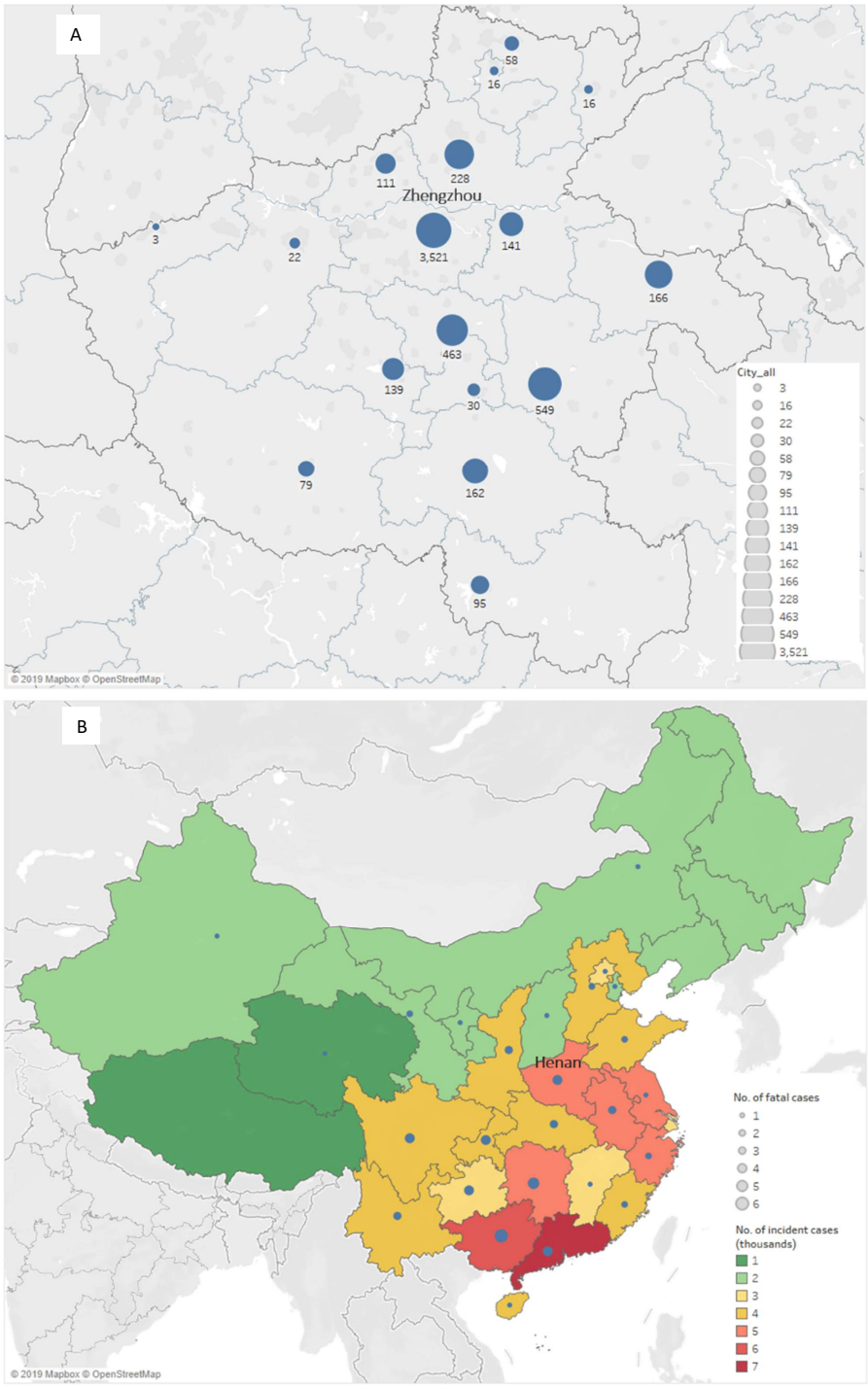


Figure 4.2: Geographical distribution of confirmed and probable HFMD cases in ZCH, 2015-2016 (n=6129)(A) The map shows the hospitalised cases with HFMD in Zhengzhou and the neighbouring cities in Henan at Admin2 (city) level. Circles indicates admitted patients who were eligible in this cohort. Each circle is proportional to the number of incident cases. (B) The choropleth map shows the incident cases and deaths from HFMD at Admin1 (province) level according to contemporary data on national HFMD surveillance in China [227].

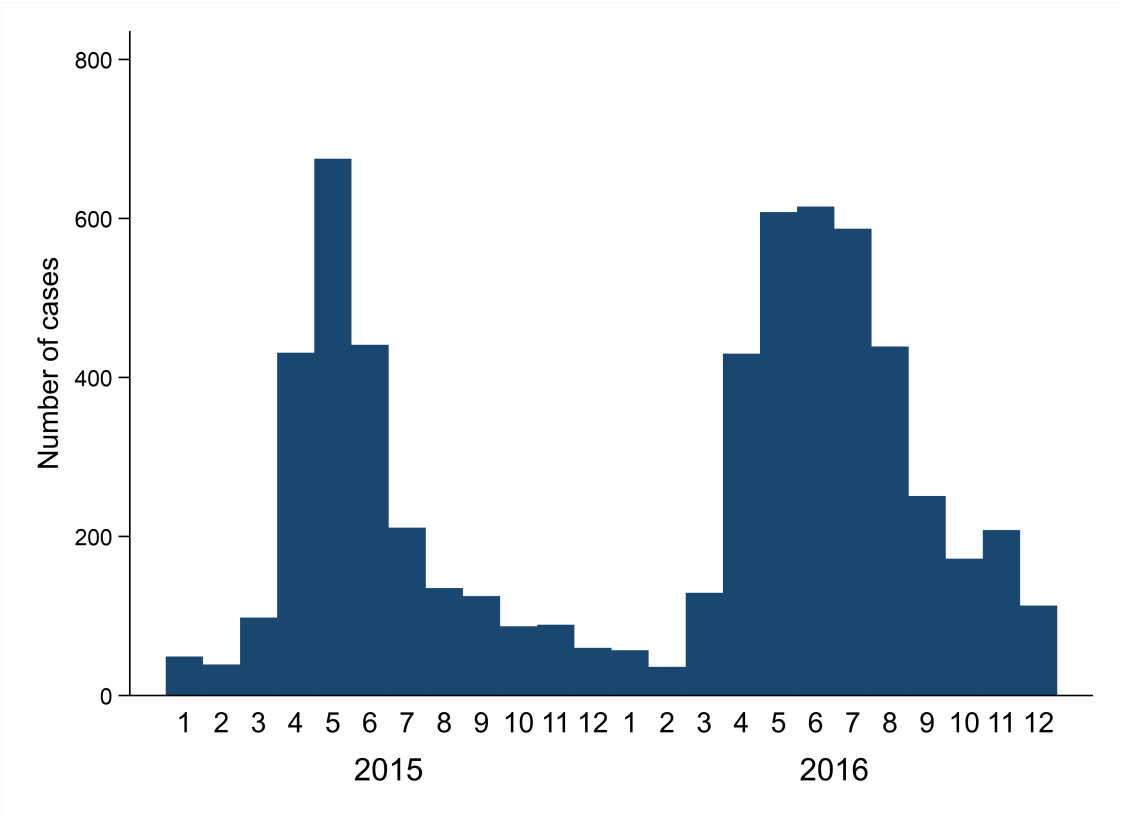


Figure 4.3: Temporal distribution of hospitalised HFMD cases in ZCH, 2015-2016 (n=6139). Numbers on the x-axis indicate month and year.

4.7.5 Demographic characteristics

The vast majority of cases were of Han Chinese ethnicity (99%). Age of the included patients ranged from 1 month to 14 years, with a median age of 1.7 years (IQR 1.2-2.5 years). 82% of patients were aged less than 3 years old. 3918 (64%) were male and the male to female ratio was 1.8. The age and sex distribution of the patients is displayed in figure 4.4.

The proportion of mothers with parity >1 was higher in the cases with severe HFMD (50% vs 40%; P<0.001). Few children (<1%) were reported has having concomitant chronic comorbidities. Around 11% of children (658/6139) had a concomitant upper respiratory tract infection, whilst 6% (368/6139) reported the presence of a concomitant lower respiratory tract infection (LRTI). A LRTI was

more commonly reported in severe cases (13%) comparted to uncomplicated cases (9%). Concomitant diarrhoea was reported in 10% (598/6139) of cases.

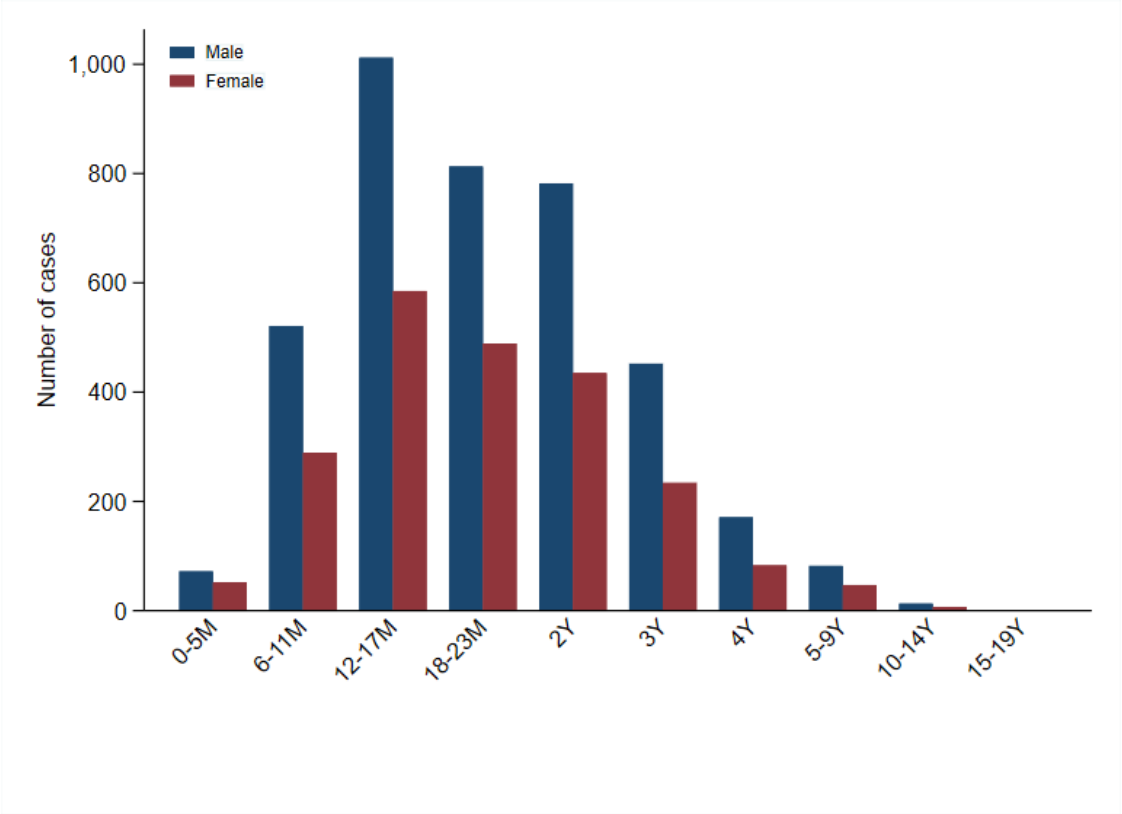


Figure 4.4: Age and sex distribution of hospitalised HFMD cases in ZCH, 2015- 2016 (n=6139).

4.7.6 Presenting and symptoms and signs

The median time from symptom onset to hospitalisation was 2 days and was slightly longer in severe cases compared to uncomplicated cases (2.8 days vs 3.6 days, P<0.001). The most common presenting signs and symptoms were fever, rash, tiredness and poor appetite (table 4.4). Forty three percent of patients were reported to have myoclonus on admission. The other most common symptoms indicative of neurological complications on admission were vomiting (22%) and limb tremor (9%). Table 4 shows symptoms and signs on admission.

	Presenting symptoms and signs on admission
Systematic symptoms	
Fever, n (%)	5942 (97.6%)
Rashes, n (%)	5825 (95.7%)
Atypical rashes, n (%)	0 (%)
Tiredness, n (%)	5591 (98.9%)
Poor appetite, n (%)	5916 (97.2%)
Gastrointestinal symptoms	
Vomiting, n (%)	1357 (22.3%)
Nausea, n (%)	99 (1.6%)
Diarrhoea, n (%)	178 (2.9%)
Neurological and musculoskeletal signs	
Headache, n (%)	51 (0.8%)
Neck stiffness, n (%)	0 (0%)
Photophobia, n (%)	0 (%)
Impaired consciousness, n (%)	111 (1.8%)
Seizures, n (%)	275 (4.5%)
Myoclonus, n (%)	2643(43.3%)
Limb tremors, n (%)	518 (8.5%)
Limb weakness, n (%)	29 (0.5%)
Decreased muscular tension, n (%)	0 (%)
Unsteady gait, n (%)	105 (1.7%)
Nystagmus, n (%)	0 (0%)
Oculomotor nerve palsy	0 (%)
Ataxia, n (%)	0 (0%)
Dysphagia	0 (%)
Dysarthria	0 (0%)
Respiratory symptoms signs	
Cough	825 (13.6%)
Tachypnea	93 (1.5%)
Abnormal breathing patterns	68 (1.1%)
Dyspnoea	14 (0.2%)
Pink froth	0 (0%)
Circulatory signs	
Cyanosis, n (%)	42 (0.7%)
Mottled skin, n (%)	6 (0.1%)
Livid skin, n (%)	7 (0.1%)
Cold limbs, n (%)	258 (4.2%)

Table 4.4: Presenting symptoms and signs on admission (n=6139).

4.7.7 Uncomplicated, mild and severe complications

Table 4.5 shows the number, and breakdown by signs and symptoms, of study subjects classified as having HFMD without complications, with mild complications, and with severe complications. Overall, 2145 (35%) were classified as uncomplicated, 3508 (57%) as having mild complications, and 486 (8%) has having severe complications. Myoclonus was the most common sign associated with mildly complicated HFMD, occurring in 2660 (76%) of the 3508 patients who did not experience any more serious complications. Sepsis was reported in 1177 (34%) of the 3508 patients who did not experience any more serious complications, but as previously reported, in this context the clinicians used the term 'sepsis' to indicate an inflammatory state rather than organ dysfunction or septic shock. Of the 486 subjects with severe complications, the most common severe complications were encephalitis (391; 80%) and ICU admission (298; 61%). ANS dysregulation was reported, in free text, by the clinicians in 62 patients. Three patients died.

Table 4.5: Key symptoms and signs among children hospitalised with HFMD.

Variables	Uncomplicated HFMD† (n_{r2}=2145) -n (%)	HFMD with mild complication (n_{r2}=3508) -n (%)	Complicated and critically ill HFMD§ (n_{r2}=486) -n (%)
History of fever OR fever on admission (>38°C) (n=5516)	1798 (84)	3274 (93)	444 (91)
History of fever (n=5380)	1755 (82)	3197 (91)	428 (88)
Fever on admission (>38°C) (n=2798)	838 (39)	1732 (50)	228 (47)
Fever on admission (≥39°C) (n=1339)	373 (17)	850 (24)	116 (24)
Fever during hospitalisation (>38°C) (n=4133)	1248 (58)	2495 (71)	390 (80)
Fever during hospitalisation (>=39°C) (n=2136)	575 (27)	1320 (38)	241 (50)
Persistent fever (n=483)	97 (5)	294 (8)	92 (19)
All rashes + diagnosis of HFMD before admission (n=6132)	2144 (100)	3507 (100)	481 (99)
Skin lesions on palms, soles or buttocks (n=6108)	2136 (100)	3496 (100)	476 (98)
Atypical rashes (sub-epidermal lesions ~1mm) (n=42)	5 (0.2)	17 (0.5)	20 (4)
Oral ulcers (n=6023)	2118 (99)	3466 (99)	439 (90)
Other symptoms and signs			
Altered level of consciousness* (n=438)	0 (0)	119 (3)	319 (66)
Coma† (n=18)	0 (0)	0 (0)	18 (4)
Headache (n=53)	20 (1)	26 (0.7)	7 (1)
Neck stiffness† (n=6)	0 (0)	0 (0)	6 (1)
Photophobia† (n=0)	0 (0)	0 (0)	0 (0)
Seizure* (n=305)	0 (0)	202 (6)	101 (21)
Myoclonus* (n=3038)	0 (0)	2660 (76)	374 (77)
Tremor* (n=671)	0 (0)	483 (14)	188 (39)
Ataxia* (n=8)	0 (0)	2 (0.06)	6 (1)
Decreased muscle tension* (n=11)	0 (0)	1 (0.03)	10 (2)
Limb weakness* (n=77)	0 (0)	26 (0.7)	51 (10)
Unsteady gait* (n=151)	0 (0)	93 (3)	58 (12)
Nystagmus† (n=2)	0 (0)	0 (0)	2 (0.4)

Table 4.5: Key symptoms and signs among children hospitalised with HFMD.
- Continued from previous page

Variables	Uncomplicated HFMD† (n_{r2}=2145) -n (%)	HFMD with mild complication (n_{r2}=3508) -n (%)	Complicated and critically ill HFMD§ (n_{r2}=486) -n (%)
Other symptoms and signs			
Oculomotor nerve palsy† (n=6)	0 (0)	0 (0)	6 (1)
Dysphagia† (n=0)	0 (0)	0 (0)	0 (0)
Dysarthria† (n=0)	0 (0)	0 (0)	0 (0)
Abnormal respiratory pattern* (n=159)	0 (0)	48 (1)	111(23)
Dyspnoea† (n=35)	0 (0)	0 (0)	35 (7)
Vomiting (n=1449)	318 (15)	886 (25)	245 (50)
Projectile vomiting (n=64)	9 (0.4)	37 (1)	18 (4)
Nausea (n=104)	30 (1)	64 (2)	10 (2)
Lip cyanosis (n=108)	2 (0.1)	30 (0.9)	76 (16)
Mottled skin (n=25)	2 (0.01)	4 (0.1)	19 (4)
Livid skin (n=28)	8 (0.4)	7 (0.2)	13 (3)
Cold limbs (n=326)	79 (4)	168 (5)	79 (16)
Cold sweats† (n=5)	0 (0)	0 (0)	5 (1)

* indicates symptoms or signs exclusively associated with any complication.

† indicates symptoms or sign exclusively associated with moderate to severe complication.

4.7.8 Demographic and other factors associated with the risk of severe complications

The mean age for patients was different in severity subgroups; with the mean age in complicated patients being younger than the mild CNS involvement group (2.0 vs 2.1, Bonferroni P=0.01). The proportion of males and females in patients was similar between subgroups. In terms of pre-existing medical conditions, more complicated cases had congenital anomalies (1.9% vs. 0.7%, P<0.001), upper respiratory tract infections (12% vs 11% and 10%, P<0.001), lower respiratory tract infections (13% vs. 9% and 4%, P<0.001), infective diarrhoea (11% vs. 11% and 9%, P<0.001), and other conditions when compared with the uncomplicated and mild CNS involved patients. The frequency of skin lesions on the palms, soles or buttocks was similar between uncomplicated and complicated cases (100% vs 98%). Although oral ulcers were reported in the great majority of cases, they were less frequently reported in complicated cases. The association between the abovementioned factors and case severity is shown in tables 4.6-4.9.

Table 4.6: Demographic factors associated with disease severity.

	Uncomplicated HFMD (n=2145)	HFMD with mild complications (n=3508)	HFMD with severe complications (n=486)	Odds ratio (95%CIs) P value
Demographic				
Age-yr				
Median (IQR)	1.7(1.1-2.8)	1.7(1.2-2.4)	1.8(1.3-2.7)	1.08 (1.00, 1.15)†
Ageband-no.(%)				
<3yr	1683(78)	2975(85)	391(80)	1.08 (1.00, 1.15)*
≥3yr	462(22)	533(15)	95(20)	
Detailed age band				
0-11M	407(19)	475(13)	47(9)	1.06 (1.01, 1.10)*
12-23M	902(42)	1764(43)	238(49)	
2Y	374(17)	736(21)	106(22)	
3Y	285(13)	346(10)	55(11)	
4Y	98(5)	129(4)	28(6)	
5-9Y	68(3)	54(2)	12(2)	
10-14	11(0.5)	4(0.1)	0(0)	
Sex				
Male sex-no. (%)	1367(64)	2221(63)	330(70)	1.22 (1.00, 1.48),
Male to female ratio	1.8	1.7	2.4	0.051
Ethnicity-no. (%)				
Han (majority)	2120(99)	3475(99)	478(98)	0.79 (0.35, 1.75),
Other minority groups	25(5)	29(1)	4(1)	0.565
Missing	2(0.1)	4(0.1)	4(1)	
Area of residence-no. (%)				
Urban	1093(51)	1636(47)	159(33)	1.91 (1.57, 2.32)*
Rural	1048(49)	1860(53)	323(66)	
Education-no. (%)				
Prior to preschool	1724(80)	3306(94)	440(91)	1.05 (0.86, 1.29),
Attendance at preschool	395(18)	202(6)	46(9)	0.604
Attendance at school	23(1)	17(0.5)	4(0.8)	

* P<0.001

† P<0.05

Table 4.7: Comorbidities associated with disease severity.

	Uncomplicated HFMD (n=2145)	HFMD with mild complications (n=3508)	HFMD with severe complications (n=486)	Odds ratio (95%CIs) P value
Comorbidities on admission-n(%)				
Congenital anomalies	14(0.7)	25(0.7)	9(1.9)	2.71 (1.30, 5.64)*
Haematological or solid malignancies	0(0)	1(0.03)	1(0.03)	11.65(0.72, 186), 0.08
Type1 Diabetes Mellitus	5(0.2)	6(0.2)	0(0)	-
Epilepsy	9(0.4)	17(0.5)	2(0.4)	0.89 (0.21, 3.78), 0.88
URTI				
Presence	243(11)	355(10)	60(12)	1.06 (1.01, 1.10)†
Absence	1818(85)	3024(86)	391(80)	
Unknown	81(4)	146(4)	30(6)	
LRTI				
Presence	183(9)	123(4)	62(13)	1.07 (1.03, 1.11)†
Absence	1733(81)	3033(86)	364(75)	
Unknown	229(11)	352(10)	60(12)	
Asthma at presentation	225(10)	317(9)	52(11)	
Presence	1838(86)	3044(87)	398(82)	1.06 (1.01, 1.11) †
Absence	79(4)	144(4)	31(6)	
Unknown				
Infective diarrhoea				
Presence	225(11)	318(9)	55(11)	1.06 (1.01, 1.11)†
Absence	1834(86)	3042(87)	395(81)	
Unknown	83(4)	145(4)	31(6)	
Other infections				
Presence	51(3)	107(3)	184(38)	1.12 (1.08, 1.61)†
Absence	1967(92)	3221(92)	256(53)	
Unknown	124(6)	177(5)	32(7)	
Other condition requiring treatment or surgery	124(6)	138(4)	110(23)	1.10 (1.06, 1.15)*
Presence	1901(87)	3200(91)	329(68)	
Absence	117(5)	167(5)	34(7)	
Unknown				

* P<0.001

† P<0.05

Table 4.8: Familial, maternal and childhood factors associated with disease severity.

	Uncomplicated HFMD (n=2145)	HFMD with mild complications (n=3508)	HFMD with severe complications (n=486)	Odds ratio (95%CIs) P value
Familial factors				
Mother's age-yr				1.00(0.99, 1.01)
Mean(SD)	29(4.8)	29(4.6)	31(4.5)	0.124
Father's age-yr				1.00 (1.00, 1.01)
Mean(SD)	31(5.3)	30(5.3)	32(5.3)	0.109
Maternal, perinatal, infant, and childhood factors				
Parity (>1)-no. (%)	813(38)	1390(40)	241(50)	1.46 (1.21, 1.76)†
Caesarean delivery-no. (%)	1132(53)	1872(54)	214(46)	0.72 (0.60, 0.87)*
Preterm (<37 weeks)-no. (%)	87(4)	99(3)	12(3)	0.99 (0.80, 1.22), 0.931
Birthweight (<2500g)-no. (%)	75(4)	107(3)	13(3)	1.07 (0.94, 1.22),
Low	1802(84)	2909(83)	379(80)	0.306
Normal	268(12)	492(14)	94(19)	
High				
Feeding choice-no. (%)				
Exclusively	1352(64)	2195(63)	308(66)	0.98 (0.96, 1.00),
breastfed	401(19)	660(19)	74(16)	0.21
Mixed feeding	373(18)	628(18)	85(18)	
Never breastfed				
Immunisation status not to date-no. (%)¶	100(5)	157(5)	37(8)	-

* P<0.001

† P<0.05

¶ Any self-reported compulsory vaccinations that were overdue by the time of hospital admission. HFMF vaccine was not approved during the study period.

Table 4.9: Physical and time-related factors associated with disease severity.

	Uncomplicated HFMD (n=2145)	HFMD with mild complications (n=3508)	HFMD with severe complications (n=486)	Odds ratio (95%CIs) P value
Physical factors				
Weight-kg				
Mean(SD)	12.9(4.9)	12.3(4.0)	12.5(3.5)	1.00 (1.00, 1.00), 0.149
Median (IQR)	12(10-15)	12(10-14)	12(10-14)	
Healthcare seeking and services				
Preadmission location				
Home	679(32)	936(27)	74(15)	-
Other facility	1466(68)	2572(73)	412(85)	
Time related factors				
Days from symptom onset to health facility-d				
Mean (SD)	2.3(2.1)	2.3(1.9)	3.1(2.5)	<0.001§
Median(IQR)	2(1-3)	2(1-3)	2(1-3)	
Days from symptom onset to hospitalisation-d				
Mean(SD)	2.8(2.8)	2.7(2.6)	3.6(3.4)	<0.001§
Median (IQR)	2(1-3)	2(1-3)	2(1-3)	
Hospital length-d				
Median (IQR)	5 (5,6)	6 (5,6)	10 (7,12)	<0.001§
ICU length-d	-	-	5.43 (4.3, 8.3)	-
Median (IQR)				

* P<0.001

† P<0.05

§ Kruskal-wallis test was used to compare median between groups instead of calculating odds ratio.

4.7.9 Vital signs

Table 4.10 shows the frequency of tachypnoea, tachycardia, hypertension and hypotension on day 1 of admission stratified by disease severity. Both tachypnoea and tachycardia are positively associated with severe disease in the univariate analysis. The odds ratio for hypertension is also high but with wide confidence intervals, reflecting the small number of patients with hypertension. Hypotension is rare and not associated with disease severity.

Table 4.10: Vital signs associated with outcome.

	Completeness -n (%)	Uncomplicated HFMD (n=2145) –n (%)	HFMD with mild complication (n=3508) –n (%)	HFMD with severe complication (n=486) –n (%)	Odds ratio (95%CI) (severe vs non-severe)	P value
Syndrome* -n (%)						
Tachypnoea	6109(99)					
Yes		565 (26)	1104 (31)	239 (49)	2.27 (1.88, 2.75)	<0.001
No		1418 (66)	2240 (64)	230 (48)		
N/A†						
Tachycardia	6108(99)					
Yes		106 (5)	242 (7)	139 (29)	6.19 (4.94, 7.75)	<0.001
No		2006 (94)	3246 (93)	339 (71)		
N/A†		20 (1)	8 (0.2)	2 (0.4)		
Hypertension	4067(66)					
Yes		2 (0.1)	1 (0.04)	39 (12)	164 (50, 534)	<0.001
No		1441 (96)	2169 (97)	286 (85)		
N/A†		54 (4)	64 (3)	11 (3)		
Hypotension	4067(66)					
Yes		54 (4)	64 (3)	11 (3)	1.08 (0.79, 1.48)	0.62
No		1441 (96)	2169 (97)	286 (85)		
N/A§		2 (0.1)	1 (0.04)	39 (12)		

* Syndromes were defined by age-related reference ranges using numeric data. They are not established by clinicians.

† These subgroups include patients with low respiratory rate, low heart rate or low blood pressure.

§ This subgroup includes patients with high blood pressure.

4.7.10 Viral aetiology

Among the 6139 clinical diagnosed patients, 5970 (97%) were tested for viral aetiology. Of these, 4772 (78%) had a positive results for either PCR or IgM or both, whereas 1198 (20%) tested negative and 169 (3%) were not tested.

The overall positive rate for any PCR was 94% (4698/4975) in stool. By comparison, the overall positive rate for any IgM was 39% (2293/5877) and 58% (148/255) in serum and in cerebrospinal fluid respectively. A breakdown of the laboratory tests and the corresponding serotype results are presented in table 4.11.

Of the 6139 included patients, 4698 (77%) and 74 (1%) were classified as laboratory-confirmed cases and probable cases, whereas 1198 (20%) and 169 (3%) had negative results for any test done or did not have a laboratory test for aetiology. The monthly number and proportion of cases by confirmed, probable, negative or not tested status are shown in figure 4.5.

Of those that had a positive PCR or IgM result, the dominant NPEV serotypes in China, EV-A71 and CV-A16, were detected in 1632 (27%) and 564 (9%) patients, respectively. Dual infections of both EV-A71 and CV-A16 was found it 54 (1%) patients. 1984 (32%) remained 'untyped' as pan-enterovirus PCR was used and the serological test did not yield any probable result for serotype. Other serotypes included coxsackieviruses detected in 514 (8%) and echovirus 24 (0.4%) patients. The results of serotyping are shown in figure 4.6.

4.7.11 Seasonality by serotype

Overall, the detected serotypes shared a similar seasonal pattern, with a single annual peak. However, the timing of the peak occurred and its intensity varied. The seasonality of admissions due to EV-A71 showed a peak in April, 2015 and

a more pronounced peak in 2016 that arrived later, in June. By comparison, the peak of CV-A16 occurred one month later than the peak of EV-A71 in 2015. But in 2016, CV-A16 had two smaller semi-annual peaks. Between 2015 and 2016, CV-A16 showed a flatter seasonal profile than EV-A71. Figure 4.7 shows the seasonality of HFMD by viral serotype.

Table 4.11: Aetiological tests and results from patients.

	Uncomplicated HFMD† (n_{r2}=2145)			HFMD with mild complication (n_{r2}=3508)			Complicated and critically ill HFMD§ (n_{r2}=486)		
Diagnostic test (n)	No. of cases tested	No. of positive (%)	95% CIs	No. of cases tested	No. of positive (%)	95% CIs	No. of cases tested	No. of positive (%)	95% CIs
Detected in any test									
Any RT-PCR or IgM assays (n=5970)	2056	1700 (83)	81-84	3432	3000 (87)	86-89	482	438 (90)	88-93
Detected in any RT-PCR in stool or rectal swabs									
EV-A71 RT-PCR (n=4975)	1661	368 (22)	20-24	2876	1075 (37)	36-39	438	243 (55)	51-60
CV-A16 RT-PCR (n=4971)	1659	290 (17)	16-19	2874	301 (10)	9-12	438	27 (6)	4-9
Pan-EVs RT-PCR (n=4971)	1659	1561 (94)	93-95	2874	2739 (95)	94-96	438	396 (90)	87-93
Any RT-PCR above (n=4975)	1661	1562 (94)	93-95	2876	2739 (95)	94-96	438	397 (91)	88-93
Detected in any IgM assays by serum									
EV-A71 IgM (n=5244)	1798	325 (18)	16-20	3011	824 (27)	26-29	435	179 (41)	36-46
Coxsackievirus IgM (n=5849)	2012	484 (24)	22-26	3364	1054 (31)	30-33	473	176 (37)	33-42
Echovirus IgM (n=5847)	2011	19 (1)	0.6-1.5	3363	33 (1)	0.6-1.4	473	3 (0.6)	0.5-3
Any IgM assays above (n=5877)	2018	637 (32)	30-34	3381	1394 (41)	40-43	478	262 (55)	50-59
Detected in any IgM assays by cerebrospinal fluid									
EV-A71 IgM (n=221)	-	-	-	-	-	-	221	48 (22)	16-28
Coxsackievirus IgM (n=251)	-	-	-	1†	0 (0)	0-98*	250	5 (2)	7-46
Echovirus IgM (n=250)	-	-	-	1†	0 (0)	0-98*	249	0 (0)	0-1.5
Any IgM assays above (n=255)	-	-	-	1†	0 (0)	0-98*	254	147 (58)	52-64

* One-sided confidence interval.

† Case was tested due to febrile convulsion.

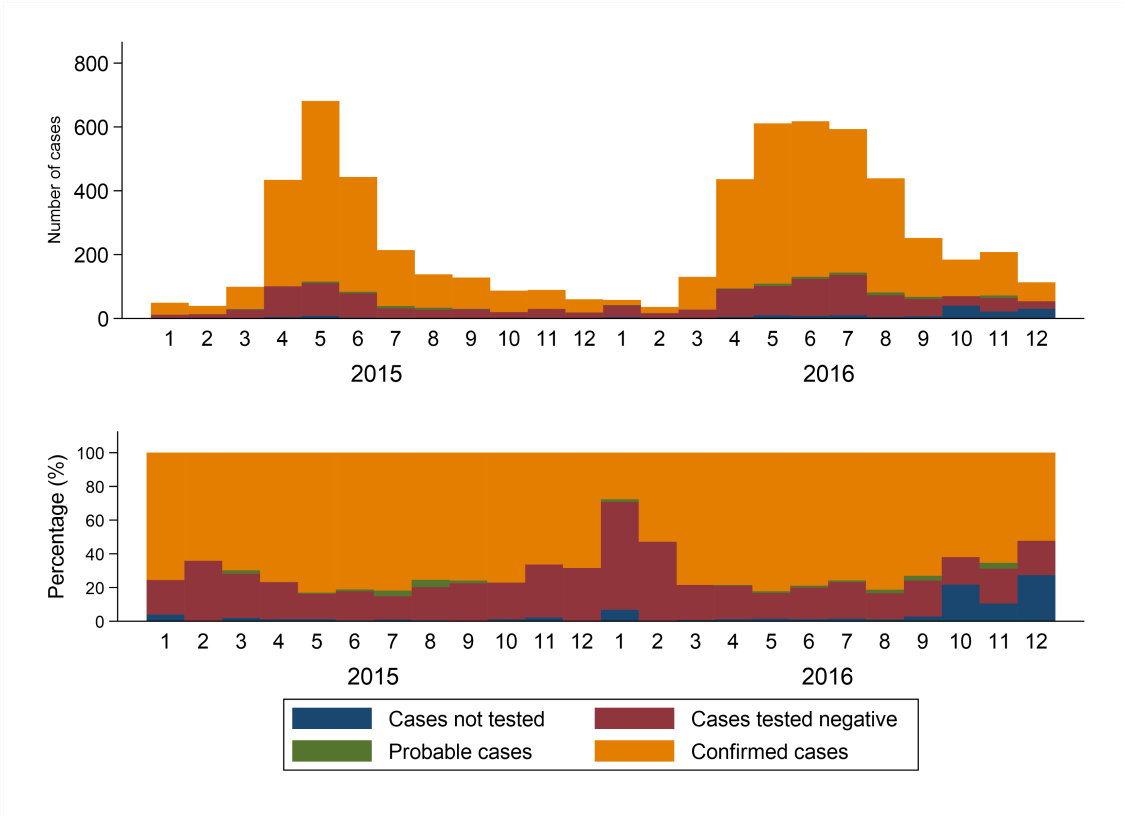


Figure 4.5: Proportion of laboratory confirmed and probable patients.

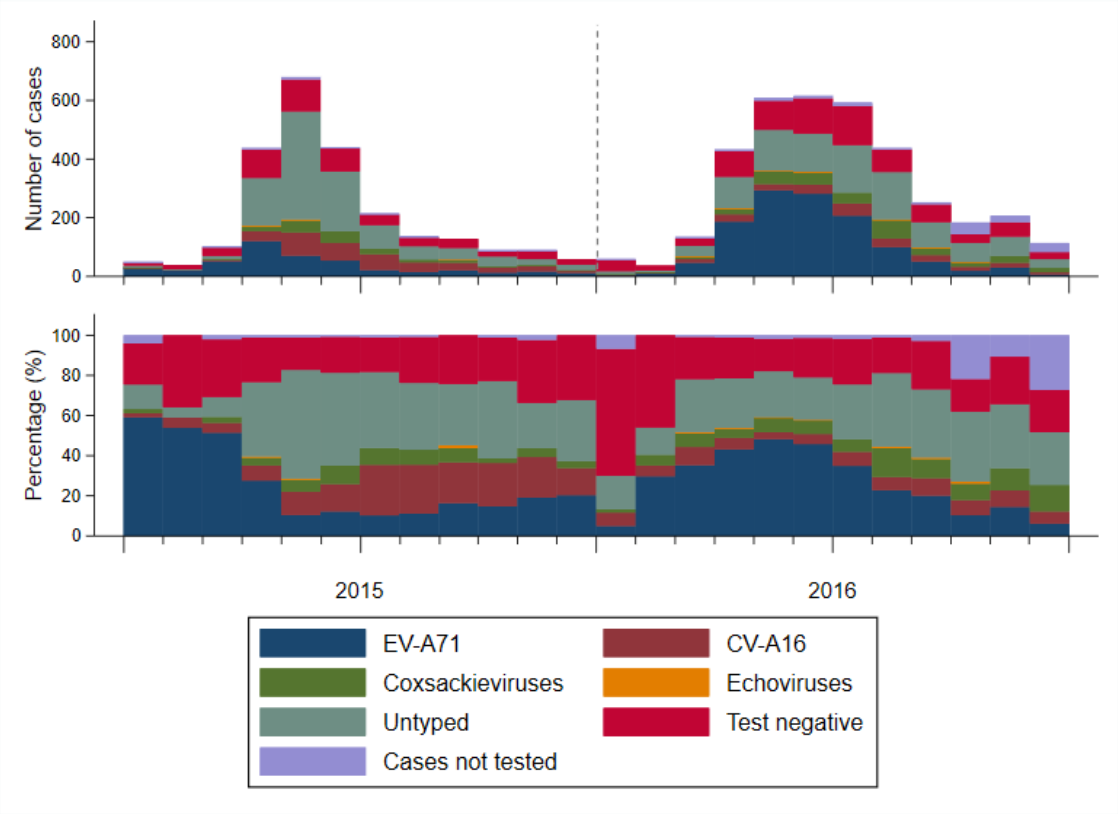


Figure 4.6: The aetiology of hospitalised patients with HFMD, 2015-2016.

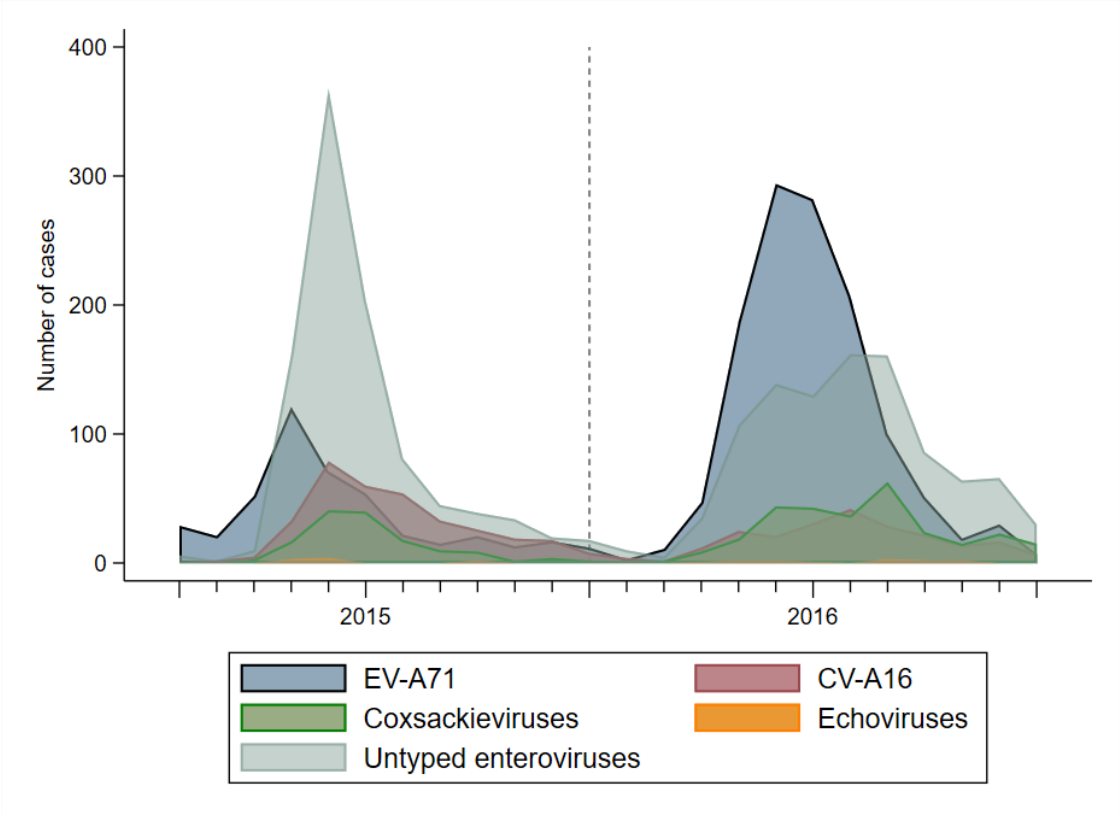


Figure 4.7: Seasonality by serotype.

Predictor variables for severe complications

For the purposes of logistic regression, disease severity was dichotomised into non-severe (uncomplicated HFMD plus mild complications) and severe disease (complicated and critically ill cases). Potential risk factors or predictors of severe disease, that were independent of the case classification criteria (table 4.5), were first assessed for association by univariate analysis (tables 4.6-4.9). These were age, gender, fever $\geqslant$39°C on admission, tachycardia on admission, tachypnoea on admission, hypertension on admission, and PCR positive for EV-A71.

The multivariate regression found that rural dwelling, EV-A71 infection, tachypnoea on admission, tachycardia on admission, and hypertension on admission were independent risk factors for severe disease (table 4.12). Age and sex were forced into the model but were non-significant. Hypertension on admission had the

highest odds ratio (98) but had wide confidence intervals due to small numbers. Tachycardia had an odds ratio of 5.42 (95% CI 3.62, 8.11) but was present in only 27% of severe cases on admission.

Table 4.12: Multivariate regression models that identified the risk factors associated with severe disease.

Candidate syndromes*	Severe disease		Univariate regression		Multivariate regression	
	Yes - n (%)	No - n (%)	Odds ratio (95%CIs)	*P* value	Odds ratio (95%CIs)	*P* value
Age						
Median, IQR	1.76 (1.33-2.67)	1.66 (1.19-2.54)	1.08 (1.00, 1.15)	0.03	0.99 (0.87, 1,12)	0.901
Sex						
Male	330 (68)	3588 (63)	1.22 (1.00, 1.48)	0.05	1.22 (0.91, 1.65)	0.189
Female (ref)	156 (32)	2065 (37)				
Rural dwelling						
Yes	323 (67)	2908 (52)	1.91 (1.57, 2.32)	<0.001	1.89 (1.41, 2.56)	<0.001
No	159 (33)	2729 (48)				
EV-A71 infection						
Yes	243 (55)	1443 (32)	2.67 (2.19, 3.26)	<0.001	1.86 (1.40, 2.48)	<0.001
No	195 (45)	3094 (68)				
Fever on admission>39℃						
Yes	116 (24)	1223 (22)	1.13 (0.91, 1.41)	0.25	-	-
No	370 (76)	4430 (78)				
Tachypnoea						
Yes	206 (47)	1210 (25)	2.71 (2.22, 3.30)	<0.001	1.42 (0.99, 2.04)	0.055
No	230 (53)	3658 (75)				
Tachycardia						
Yes	139 (29)	348 (6)	6.19 (4.94, 7.75)	<0.001	5.42 (3.62, 8.11)	<0.001
No	339 (71)	5252 (94)				
Hypertension						
Yes	39 (12)	3 (0.01)	164 (50, 534)	<0.001	98 (13, 752)	<0.001
No	286 (88)	3610 (99.9)				
Hypotension						
Yes	11 (4)	118 (3)	1.08 (0.79, 1.49)	0.61	-	-
No	286 (96)	3610 (97)				

* Syndromes were defined using age-related reference ranges and numeric data. They are not established by clinicians.

4.7.12 Clinical Management

Table 4.13 summarise the most common medicines given to patients, stratified by severity. Mannitol and methylprednisolone were given to 66% and 76% of patients with mild complications respectively. Patients with severe complications were treated with methylprednisolone (90%), mannitol (88%), IVIG (67%), and furosemide (64%). Inotropes were also commonly given to patients with severe complications. A significant proportion of patients with uncomplicated HFMD received steroids, with 36% receiving methylprednisolone.

Table 4.13: Treatment and outcomes.

Treatment	Number of patients	Uncomplicated HFMD (n=2145) –n (%)	HFMD with mild complication (n=3508) –n (%)	HFMD with severe complication (n=486) –n (%)
Medication -n (%)				
Mannitol	2886	135 (6)	2322 (66)	429 (88)
Furosemide	697	64 (3)	322 (9)	311 (64)
IVIG	609	15 (0.7)	279 (8)	325 (67)
Dexamethasone	1011	204 (10)	622 (18)	185 (38)
Methylprednisolone	3852	764 (36)	2651 (76)	437 (90)
Milrinone	69	-	-	69 (14)
Dobutamine	45	-	-	45 (10)
Dopamine	96	-	-	96 (20)
Epinephrine	105	-	-	105 (22)
Norepinephrine	4	-	-	4 (1)
Outcome -days				
Length of stay – Median, IQR	6137	5 (5-6)	6 (5-6)	10 (7-12)

4.8 Discussion

This chapter reports the findings from one of the largest and most complete clinical and laboratory datasets ever assembled of children hospitalised with HFMD. The dataset comprised over 500,000 values and had a high level of completeness. Public awareness of the HFMD outbreaks has led to increased recognition, and an evolving clinical picture of disease severity at presentation. This work systematically presented the demographics, epidemiology, clinical features, management and outcomes of 6139 paediatric patients admitted in 2015-2016. Clinical severity was associated with different NPEV serotypes, including EV-A71 and other non-EV-A71 enteroviruses. Although milder disease at presentation was associated with non-EV-A71 infections and shorter length of hospitalisation, non-EV-A71 infections also lead to severe forms of diseases. Multiple neurological syndromes and signs for autonomic instability could be recognised as unique clinical features to differentiate complication associated with NPEVs. Of these, tachycardia on admission could be a predicting factor of severe cases.

The demographic characteristics were largely consistent with previous findings in Asia and the US [206, 241]. The study population had a median age of 1.7 years (IQR 1.2-2.5 years). Children aged under 5 years constituted 97% (5990/6139) of all included cases and those aged between 12 and 23 months constituted almost 50% of the entire cohort (2904/6139). Similar median age of 2 years (IQR 1.4-3.4 years) was reported in a prospective study of 555 outpatients at the same study site [242]. This indicated that EVs causing HFMD were highly transmissible among young children [32]. Although young children appeared to be the large majority of the hospitalised cases, this confirmed to other studies that have analysed age-specific incidence data, such as national surveillance data of HFMD from China CDC, with a proportion of 90% in 2015 and 2016 [206, 243]. As has been previously reported in other clinical cohorts, there was a preponderance of males (64%; 3918/6139). The male-to-female ratio was 1.8 in all cases and 2.1 in cases with severe complications, indicating that

sex was a risk factor associated with severe forms of disease. Severe cases accounted for 8% of all included cases, which was higher than the estimates from national surveillance data [32]. This was because the study site was a tertiary hospital; therefore, patients with severe complications were more likely to be admitted to the hospital. Most children had been previously healthy and had updated immunisation status. Those who had severe complications were more likely to reside in rural area.

Overall, the seasonality of admissions showed a single annual peak, which is consistent with the national surveillance data on HFMD in China. A large proportion of cases were tested for the presence of NPEV and were positive for EV-A71 or CA-A16 or were positive in a pan-enterovirus assay. Overall, the proportion of laboratory confirmed case was >90%, as opposed to that of 3.7% in the surveillance between 2008 and 2012 [32]. Although detection of RNA in stool does not prove causality, the findings are consistent with what is known about the aetiology of HFMD generally and in China. The predominant detected NPEV serotype was EV-A71 in both years but in 2015 a large proportion of detections were through a pan-enterovirus PCR and were not typed partly because other serotype-specific diagnostic tests were not available and other EV-As could be circulating in a cyclical pattern [23]. CV-A16 was the second most common serotype detected. This is consistent with the reported epidemiology of HFMD in China [**Liy2020**, 206]. Contemporary studies reported CV-A6 was an emerging as the most common type in other cities in China, while another prospective cohort in the same city reported CV-A4, CV-A10 and CV-A6 as the most common EVs in spite of cyclical and geographical variations [**Liy2020**, 206].

Of those who had a positive result for any aetiological test for an individual EV type (4772/5970), EV-A71 and CV-A16 were detected in 27% and 9% of all these patients only, while other non-EV-A71, non-CV-A16 constituted at least 41%. More specifically, EV-A71 was detected in 50% of cases with severe complications and in 25% of cases with no or mild complications (data not shown). Among cases

with severe complications, EV-A71 constituted 50%, surpassed CV-A16 (5%) and other non-EV-A71, non-CV-A16 EVs (29%). Among uncomplicated and mildly complicated cases, EV-A71 accounted for only 25%, while CV-A16 and other non-EV-A71, non-CV-A16 EVS composed of 10% and 43%, respectively. EV-A71 predominated particularly in severe cases, this was consistent with other reports [32, 241]. The findings was also in agreement with other studies reporting the replacement of predominant NPEV types [32, 206].

Early recognition of HFMD outbreaks caused by EV-A71 could help frontline healthcare providers to recognise clinical features differentiating neurological complications due to EV-A71 from other clinical syndromes. Clinical syndromes that could distinguish uncomplicated cases from complicated ones included impaired consciousness, myoclonus, seizure, and tremor. These symptoms are important clinical features that differentiating neurological complication caused by EV-A71 from other diagnoses. Of note, myoclonus was a very frequent sign, being reported in 43% of patients on admission. The prevalence of myoclonus was 76% and 77% in mild and severe complicated cases, respectively. This sign was also largely responsible for the categorisation of 57% of patients (3508/6139) as HFMD with mild complications. However, it was not always suggestive of a severe complication, which was inconsistent with earlier reports of cases with neurological diseases in Taiwan [244, 245]. When these symptoms were present in the setting of a febrile illness, suspicion for neurological disease associated with NPEVs should be warranted to avoid delayed diagnosis [241].

I categorised patients into three severity classes based on a combination of signs and symptoms and medical interventions (e.g. ICU admission). This was necessary in order to evaluate risk factors for disease severity. We developed out own method since the existing case classification systems are rather ambiguous in how they should be applied (see chapter 5). Overall, 35% had uncomplicated disease, 57% had mild complications, and 8% had severe complications. There was

a clear relationship between our severity classification and the medical interventions used (which were not included in the classification criteria). For example, 66% of cases we classified as having mild complications were treated with mannitol and 76% were treated with methylprednisolone, suggesting that our classification correlated with the clinician's impression of disease severity. The majority of patients were managed as complicated cases, reflecting a cautious approach in the day to day management practice in the hospital. Amongst the complicated and critically ill patients > 60% (298/486) needed ICU admission and 25% (125/486) were ventilated. However, overall mortality was very low with only three deaths. We found high rates of steroid use (dexamethasone and methylprednisolone) in both uncomplicated cases and cases with mild complications, although we were not able to quantify the doses used. IVIG was used in 10% of patients, mostly in the severe group, of whom 67% received IVIG. The value of both steroids and IVIG in HFMD is unclear. Only one clinical trial reports randomisation of patients to IVIG and steroids [246] and although it reports a benefit, the results of statistical analyses are not reported in the abstract and it has not been possible to obtain the full text of this manuscript to assess the quality of evidence.

Mannitol, which is used to manage cerebral oedema and raised intracranial pressure, was given (2886/6139) to 47% of cases. The clinical indications for this practice are unclear but it certainly warrants further investigation. According to the Chinese HFMD case management guideline, mannitol is suggested for treating patients with a presumptive diagnosis of neurological complication. Given the high frequency of myoclonus in the group with mild complications, it is possible that myoclonus is being perceived as a sign of raised intracranial pressure and therefore an indication for mannitol, and perhaps also steroids. However, the significance of myoclonus as an indicator of CNS involvement and raised intracranial pressure in HFMD is unclear.

In univariate analysis we found that many variables were statistically associated

with disease severity (tables 4.6-4.9) but the effect size (odds ratio) was often very small, indicating that despite statistical significance due to the very large sample size, many of these factors had little clinical significance. We did not find an inverse relationship between the presence of the classical rash on palms, soles and buttocks with severe disease that has previosuly been reported by Hoang et al and Crabol et al [213].

In multivariable analysis, we found that tachycardia on admission was significantly associated with a risk of developing severe disease, with an odds ratio of 6.19 (95%CI: 4.94, 7.75). After adjusting for age and sex, this result confirms evidence of an association between increased age-adjusted heart rate with severe disease, meaning the risk of severe disease among a child with increased age-adjusted heart rate is around 5 times higher than those with a normal heart rate. Our results also confirm the previously reported association of EV-A71 infection and severe disease, with an adjusted OR of 1.86 (95%CI: 1.40, 2.48). Hypertension on admission shows a very high OR, this may be partly explained by the fact that hypertension is the hallmark of incipient cardiopulmonary failure. Nevertheless, hypertension on admission was absent from the majority of patients who had severe disease, so whilst it may have a good positive predictive value, it would have a poor negative predictive value.

One strength of our study is that it described a complete cohort of admitted paediatric patients over a full two year period. Since all patients admitted over a two-year period were included, there is a low risk of selection bias. Data extraction was very comprehensive and resulted in a very large dataset which included daily clinical symptoms and signs and had a high level of data completeness. This was related to the use of standard admission and discharge pro formas. For complications noted by the clinicians, data were extracted from both fixed and free text fields. Nevertheless, this study is based on electronic medical notes and suffers from incompleteness and difficulty in interpretation of information [247]. For example, it

was not possible to verify whether the clinician diagnosis fully met all the criteria of the WHO definitions for these complications.

To minimise the impact of recognised limitations of retrospective case not reviews, the following methodological considerations were made: 1) research questions were formulated and refined during the planning stage; 2) study site and sampling strategy were decided in consideration of the sparse complicated cases, the extremely low CFR and the periodicity of epidemics; 3) proposal was developed to provide a standardised way for data collection; 4) adequate variables that can be operationalised were collected; 5)data extract operators were trained and monitored; 6) standard procedure operations were created to ensure consistency during the conduct of the study; 7) [221, 222].

There were also some limitations in this work. Risk of selection bias could not be ruled out, due to the selection of a single study site and possible detection bias caused by vigilant healthcare providers [248]. For example, 97% of the children were younger than 5 years old, which could compromise the representativeness of a population of older than 5. We were not able to have a complete record of outpatient visits to determine why the older children were not admitted, which was one of the limitations of a retrospective study. However, the results should have representativeness of the younger population aged less than 5, especially considering the wide range of clinical outcomes and severity.

In this study, ANS dysregulation was a severity-defining variable. This would yield a more precise estimate for disease burden and case characterisation. However, in regression models, predicting factors such as tachypnea and tachycardia were part of the definition of severity. This constitutes at least partially a circular argument, which was a limitation of the analyses. Future advanced analyses should select more objective laboratory parameters, such as CSF pleocytosis. In the multivariate analyses, risk factors with predictive of disease severity were selected. Due to the

time limit, the validation of predictive model was not performed. This could have been done by separating the data into a testing and a validation dataset.

Finally, as in any observational study, confounding may mask the an actual association, or demonstrate a spurious association, which should be controlled in epidemiological studies [249]. We identified confounders that were reported in other studies, such as age, sex and EV-A71 infections. Although we adjusted for multiple potential confounders, residual confounding may persist and findings should be interpreted with caution.

5

Assessment of clinical case classification criteria systems for HFMD

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5.1 Preface to the chapter

This chapter seeks to evaluate different national and international clinical case classification systems for HFMD. Major clinical case classification systems are identified through a literature search and then applied to the retrospective cohort dataset described in the chapter 4. The agreement of the clinical case classification schemes to identify patients requiring intensive medical management is assessed. This chapter meets objective 4 of the thesis: to evaluate the usefulness of the existing HFMD clinical case classification systems and make recommendations on their use and further developments.

5.2 Author's contribution

I designed and implemented all the work described in this chapter. This included performing the literature search to identify the relevant clinical case classification systems; adapting the severity schemes for analysis; assembling, cleaning, validating and reconciling the cohort database for analysis; applying the case classification schemes to the cohort data; performing the comparative analysis and all other statistical analyses. I drafted all the text and produced all figures and tables.

5.3 Abstract

Background: Multiple clinical case classification systems for HFMD have been developed since the first outbreaks of EV-A71 with severe outcomes were recognised in Malaysia in 1997. However, the performance of these case classification systems has not been evaluated.

Objective: This study aims to evaluate these systems for their usability, agreement, and their ability to correctly discriminate between mild and severe cases.

Methods: HFMD clinical case definition systems were identified through a literature review. Two case classification systems, the WHO and Vietnamese, were applied to data from a retrospective cohort of 6,139 patients enrolled from January 1, 2015 to December 31, 2016 in Zhengzhou, China. Construct validity, the ability of the tools to measure what they claim to measure, was assessed by evaluating the ability of the two systems to correctly classify 'severe cases' as defined by the level of medical intervention they required (composite: all mortality or ventilation use or inotropes use or ICU admission). Agreement between the two case definition systems was assessed using the Cohen's Kappa coefficient. Performance was assessed by calculating the area under the receiver operating characteristic curve (AUROC).

Results: Upon application of the WHO classification scheme to the clinical dataset, 99% (5762/5810) of the cases were in agreement with the non-severe case definition but only 42% (124/294) were in agreement with the severe case definition. The positive predictive value was 72% (95%CI 65-78%) whilst the negative predictive value was 97% (95%CI 96-97%). In comparison, applying the Vietnamese system, 90% (5256/5825) of the cases agreed with the non-severe case definition and 84% (248/294) of the severe case definition. However, the positive predictive value of the Vietnamese classification was only 30% (95%CI 28-32%) because the Vietnam system had low specificity and classified a large number of false positive severe cases. The Vietnamese system was better able to identify severe cases using the AUROC (AUROC 0.87 95%CI 0.84, 0.89) than the WHO system (AUROC 0.85 95%CI 0.83, 0.87). There was only 'fair' agreement between the two schemes.

Conclusion: Among those diagnosed with HFMD, both the Vietnamese and the WHO schemes had limitations, with the WHO scheme suffering from poor sensitivity and the Vietnamese scheme suffering from poor specificity. We also identified ambiguities in the schemes, that if corrected would probably improve their

utility. Further research through prospective studies is needed to better establish the usability of these tools and their agreement.

5.4 Background

HFMD has become the most prevalent viral disease among young children in Southeast Asia since the early 2000s. It is caused predominantly by *Enterovirus* A members, of which EV-A71 and CV-A16 are most commonly reported. Of the children with HFMD who come to the attention of medical staff, the most common clinical manifestation is a non-febrile illness with classic exanthema on palms, soles, buttocks or posterior oral cavity. Less frequently, HFMD patients can develop severe complications. Citywide data from Shanghai from 2007 to 2010 showed that amongst 28,058 outpatients with HFMD around 14% of cases were hospitalised, 2.6% developed more severe forms of disease, and 0.04% died [250]. Nationwide data from the infectious disease reporting system in China shows a lower risk of developing severe complications at around 1% [251].

In the infancy of the Asian epidemic, several investigations were undertaken to report and describe severe HFMD and to characterise patients who progressed rapidly to cardiopulmonary failure and death [244, 252]. These early papers reported the onset of pulmonary oedema or haemorrhage preceded by neurological complications such as myoclonus, tremor and ataxia [244]. It was postulated that the pathogenesis of pulmonary oedema and haemorrhage was associated with lesions in the spinal cord and medulla, which resulted in an excessive release of catecholamine and cortisol that disturbed the autonomic nervous system and led to neurogenic shock and pulmonary oedema [245, 252, 253]. An alternative hypothesis was that systemic viral sepsis led to a capillary leak syndrome, which accounted for the observed hyperglycaemia, metabolic acidosis and shock [253].

In response to these very severe complications, Taiwanese investigators were the first to formally define clinical stages with the objective of improving case management and clinical outcomes. The earliest classification was: stage 1 as HFMD only; stage 2 as encephalomyelitis, stage 3A as hypertension; stage 3B as hypotension; and stage 4 as convalescence [245].

In view of the growing incidence of HFMD, WHO, the Vietnam Ministry of Health and the China Ministry of Health (MOH) developed their own clinical case classification systems, with the aim of improving case management [33, 205, 254–256].The WHO classification scheme was developed in 2011 and the Vietnamese and Chinese national classification schemes in 2012 and 2018 respectively by expert consensus.

5.5 Rationale for investigation

Ideally, a good HFMD classification scheme would consistently and correctly classify patients and guide appropriate clinical management, including the use of interventions such as IVIG. Also, a standardised and reliable severity assessment tool can help with comparative clinical epidemiology and the implementation and interpretation of clinical trials (result in marked discrepancy in surveillance data and expecting studies). In addition, to be effective, a scheme needs to be simple and reproducible, use-friendly and applicable throughout health-care systems of the countries where it is to be used. However, the abovementioned clinical case classification systems are, at face value, somewhat discordant and ambiguous. Furthermore, some signs used in the classification systems can have different clinical severities. This is exemplified by myoclonus, which can range in severity from mild myoclonus during sleep to persistent myoclonus during waking hours [244]. If such indicators are not well defined, the validity of the severity classification tool may be reduced.

The importance of critically appraising case classification systems can be exemplified by dengue. The WHO dengue case classification system which defined dengue fever, dengue haemorrhagic fever and dengue shock syndrome was subjected to substantial evaluation. It was found that the old system had significant limitations, being difficult to apply, with a large proportion of patients being unclassifiable, and having low sensitivity in classifying DHF [257–259].This led to a major revision of the classification system in 2009, which itself has been subjected to rigorous evaluation [260–263].

However, to date there has been no critical review or comparison of HFMD clinical case classification schemes. Herein, the existing classification schemes are reviewed and a retrospective analysis is performed to evaluate their utility.

5.6 Aim and objectives

The overarching aim of this work is to describe the different HFMD clinical case classification schemes and evaluate them by applying them to a large clinical database. The first objective of this work was to provide a narrative description of the usability of the schemes. The second objectives was to report the application of different schemes to a clinical dataset. The third objective of the work was to assess the concordance of the different schemes to discriminate between mild and severe cases. The fourth objective was to make recommendation on a standard severe case definition to facilitate case classification and clinical research in the future.

5.7 Materials and methods

5.7.1 Literature search

Literature search

To identify existing severe HFMD classification schemes and the corresponding management guideline, a literature search was performed. PubMed was search from January 1, 1998 onwards to June 1, 2019. The time frame was specified as the occurrence of HFMD with severe forms was not common until the outbreak in Malaysia and Taiwan in 1998 [33, 244]. The search term was as follows: ((hand foot mouth disease OR HFMD OR hand-foot-mouth disease) AND (severe OR complicated OR complication OR death OR fatal)) OR (stage-based management). This was supplemented by manual search for existing severity schemes or treatment guideline in Google Scholar. The Vietnamese classification scheme was requested from Oxford University Clinical Research Unit in Hanoi, Vietnam. No language restriction was imposed. The title and abstracts were reviewed by a single reviewer to identify potentially relevant HFMD case classification schemes.

Selecting severity schemes

A severity classification scheme was reviewed and assessed if it (1) clearly stated the elements (syndromes or parameters) for the severity criteria; and (2) incorporated guidance on case management. A scheme was reviewed but not assessed if it was redundant, incomplete, not clearly stated or did not provide any information on case management.

Summary of guidelines and schemes

To present a summary of the candidate guideline on severity classification and case management for comparison and analysis, a standardised form was used to collect the information as follows: name of authority, country, publication year, severity grades, and the criteria or elements for severity grading.

Deriving a cohort for validation

A cohort of clinically diagnosed HFMD cases was assembled as described in Chapter 4 (section 4.7).

5.7.2 Classifying patients using the existing schemes

Each case was classified according to the WHO and Vietnamese schemes by applying the components of the severity scheme to clinical data recorded in the first 48 hours after admission. Unspecified clinical indicators such as 'tachypnoea', 'hypertension' and 'hypotension' were classified based on age-specific normal ranges (source Royal College of Nursing). It was not possible, due to time constraints, to code the Chinese scheme. The maximum grade for each severity scheme over the 48-hour period was used. Since details on the absolute frequency of myoclonus and whether it was carer or physician observed was not systematically available in the dataset a modified version of Vietnam guideline was used in which grades 2a and 2b were distinguished by 'frequent myoclonus' (grade 2b) or infrequent or unspecified frequency of myoclonus (grade 2a).

To assess concordance of the schemes in identifying severe HFMD, a binary division on severe and non-severe was also created, where severe was defined based upon the treatment recommendation that ICU admission was required; this equates

to grades 2b, 3 and 4 in the Vietnamese system, and grades 3 and 4 in the WHO system (tables 5.3 and 5.4).

5.7.3 Establishing reference severity based on clinical intervention level

We assessed the agreement of the classification schemes using the level of clinical intervention required as a proxy for severity. This methodology has previously been used by Narvaerz et al to evaluate dengue severity classification [264]. A severe case of HFMD was defined as a patient with HFMD who either died and/or required ICU admission.

5.7.4 Ethical approval

The study was approved with waiver of informed consent by OXTREC, Ethics committees of China CDC, and Zhengzhou Children's Hospital (ZCH).

5.7.5 Statistical analyses

The proportion of HFMD cases that were classifiable and their classification using the different schemes is presented. The level of agreement between existing schemes for the detection of severe cases of HFMD was determined using Cohen's kappa coefficient. A Kappa value, 0.00 was considered "poor agreement", 0.00–0.20 as "slight agreement", 0.21–0.40 as "fair agreement", 0.41–0.60 as "moderate agreement", 0.61–0.80 as "substantial agreement", and 0.81–0.99 as "almost perfect agreement' [265].

The agreement of the schemes in classifying severe HFMD cases was further evaluated by calculating the area under the curve (AUC) of the receiver-operating

characteristics (ROC) curve. By convention, an AUC between 0.5 and 0.7 indicates a poor discriminative capacity; an AUC between 0.7 and 0.9 indicate a reasonable capacity; an AUC > 0.9 indicate a very good capacity. Statistical analyses was performed using Stata (version MP 15.0).

A number of sensitivity analyses were performed, wherein the AUROC for the outcome measures were calculated in a range of scenarios. These include: (1) fatal versus survived patients, (2) mechanical ventilated versus non-ventilated patients, (3) inotropes used versus no inotropes used, (4) ICU admitted versus non-ICU admitted, (5) length of ICU stay >3days, (6) length of ventilation use > 3days; (7) prolonged hospital stay > 7 days and non-prolonged stay.

5.8 Result

5.8.1 Literature review

The literature search yielded a 1029 publications within the specified time frame. In total eight publications that described a clinical case classification scheme for HFMD were identified. An overview of these eight publications is shown in table 5.1. Three publications from Taiwan were excluded from further analysis since these were superseded by subsequent publications from WHO and Vietnam, which were adapted based on them. The Malaysian and Indian guidelines were excluded as they did not incorporate any case management guidance. Three clinical case classification schemes were therefore included for further analysis of concordance and performance, these were the WHO, Chinese and Vietnamese systems.

Table 5.1: Overview of the HFMD guidelines/publications and case classification systems.

Scheme names	Year	Country	Classification of authorising body	Authorising body	Condition	Target population	Intended use/user	Language
1. Grades of rhombencephalitis	1999	Taiwan (China)	N/A	Huang1999, Research paper (NEJM)	EV-A71 infections with neurological complications	Children	Unspecified	English
2. Clinical stages and management of EV71 infection	2002	Taiwan (China)	N/A	Lin2002, Research paper (CID)	EV-A71 infection with signs and symptoms of CNS involvement	Children	"Clinical stages and management of EV71 infection"	English
3.EV71 stage-based management	2004	Taiwan (China)	Regional	Chang2004, Research paper (PIDJ); Centre for Disease Control in Taiwan	EV-A71 infections	Children	"Clinical stage and disease management of these stages"	English
4.EV-A71 guidelines	2007	Malaysia	National	Malaysian Ministry of Health	Symptomatic HFMD cases	Children and infants	"Health personnel and health authorities"; "Detect and control epidemics, strengthen emergence response capacity, improve outcome of HFMD cases"	English

Table 5.1 Overview of the HFMD guidelines/publications and case classification systems. - *Continued from previous page*

Scheme names	Year	Country	Classification of authorising body	Authorising body	Condition	Target population	Intended use/user	Language
5. HFMD	2008	India	National	Indian National Institute of Communicable Disease	HFMD cases	Children	"Response in case of an outbreak"	English
6. Chinese guideline for diagnosis and treatment guideline on HFMD	2008, 2010, 2018	China Mainland	National	Chinese Ministry of Health	HFMD cases	Children	"Diagnosis and clinical management"	Chinese, English
7. A guide to clinical management and public response for HFMD	2011	N/A	International	World Health Organisation	HFMD cases	Children	Unspecified; "Standardised case definition and guideline for clinical management of severe cases of EV71- associated HFMD"	English
8. Flow chart for diagnosis and treatment of HFMD in children	2011	Vietnam	National	Department of Health	HFMD cases	children	"Diagnosis and treatment"	English

5.8.2 Descriptive summary

In 1999, a severity grade was first proposed among 41 children with neurological complications associated with EV-A71 infection in Taiwan [244]. Transient signs of brainstem encephalitis, such as myoclonus, tremor and ataxia were observed in three patients before the onset of acute flaccid paralysis. Three severity grades were suggested, with grade I defined as generalised myoclonus with tremor, ataxia or both, grade II as myoclonus with cranial nerve involvement, and grade III as rapid cardiopulmonary failure (rapid onset of respiratory distress, cyanosis, poor peripheral perfusion, shock, coma, loss of the doll's eye reflex and apnoea). However, the application of this grading scheme was limited to cases with neurological complications. In grade I and II, abnormal EEG results presented in 10% and 30% patients respectively, and chest x-ray revealed no lung abnormalities in contrast to grade III who had diffuse pulmonary oedema and pneumonic infiltration and higher lactate level in CSF. Grade III encephalitis was considered to be potentially fatal, complicated by acute cardiopulmonary failure and extensive brainstem damage, with the dorsal pontine tegmentum the most commonly affected area.

A stage-based clinical management approach was first reported in 2002 [245]. The scheme had five levels: (1) HFMD/Herpangina; (2) encephalomyelitis; (3a) cardiopulmonary failure with hypertension; (3b) cardiopulmonary failure with hypotension; and (4) convalescence. The approach recommended that at stage 2, patients with signs of increased intracranial pressure should be given osmotic diuretics and furosemide if they were suspected to have fluid overload. Anticonvulsant medication was recommended to control seizures. Blood pressure, oximeter readings and blood sugar should be closely monitored. At stage 3, hypertension was considered as the hallmark characteristic, with cold sweating, hyperglycaemia and frequent myoclonic jerks. Continued fluid restriction was suggested as well as the use of vasodilators (nitroprusside and milrinone) to control severe hypertension. Early intubation allowed positive-pressure mechanical ventilation with increased

positive end-expiratory pressure for treatment of pulmonary oedema. Use of a high-frequency oscillatory ventilator should be considered for pulmonary oedema or haemorrhage persists or severe hypoxemia develops. At Stage 3B, hypotension could occur and nitroprusside should be discontinued and inotropic agents (dopamine and epinephrine) become necessary to maintain sufficient perfusion pressure. The use of ECMO or left ventricular assist device still remained controversial.

This stage-based management was evaluated in a before and after study design which compared 196 HFMD patients with CNS involvement and cardiopulmonary failure who did not have stage-based management (1998 to 1999) to 331 patients who did (2000-2002) [266]. Although subject to limitations, such as small sample size and inclusion of complicated patients only, the effectiveness of this stage-based management was reported as being substantial, with case fatality during the acute phase decreasing from 83% (15 of 18) among those who received conventional treatment to 33% (12 of 36) among those received stage-based management [266].

The first national guidelines were introduced in 2007 in Malaysia but case classification was limited to EV-A71 infections. [267]. Although the guideline provides clinical case definitions for HFMD and the associated myocarditis, meningoencephalitis or acute flaccid paralysis, the purpose of the case classification was for laboratory use (suspected and confirmed) and focuses primarily on public health responses and surveillance instead of clinical management. Similar guidelines were also introduced in India.

The first national clinical management guideline was developed in China in 2010. In this guideline, both a case definition and severity grades were defined and clinical management suggested. HFMD cases were classified into four stages and three categories: mild (stage 1), severe (stage 2) and critically ill (stages 3 and 4) (table 5.2). In 2012 the Vietnam Ministry of Health developed a national stage-based clinical management guideline based on the Taiwan guidance and included 5 categories [256]. An adaptation of the full Vietnamese guideline is shown in table 5.2.

The WHO guideline was developed by expert consensus in order to identify severe cases and to provide prompt case management. This guideline specifies presumptive diagnosis, warning signs and four stages: uncomplicated HFMD/HA; HFMD with CNS involvement; HFMD with autonomic nervous system (ANS) dysregulation; and HFMD with cardiopulmonary failure (table 5.3) [33].

In terms of the severity grades, there is an agreement across all three schemes of using skin lesions on hand, foot and mouth and/or oral ulcers without complications to define case as mild or uncomplicated HFMD. The three schemes are also in agreement in defining two general types of clinical complication; neurological and cardiopulmonary. The Vietnamese scheme differs from the WHO and Chinese in that it stratifies CNS involvement into mild and severe, thereby having five rather than four classes. The WHO guideline (pages 39 to 42), uses the terms 'severe complication', 'severe case' and 'severe disease'. On page 40, severe disease was said to be characterised by three distinct stages: CNS involvement, ANS dysregulation, and those with frank cardiopulmonary failure, including pulmonary oedema or haemorrhage. In addition, those presenting with neurological signs but without signs of ANS dysregulation (persistent resting tachycardia, hypertension or profuse sweating) can be considered to be in the CNS involvement stage (early stage of severe disease), which can manifest as aseptic meningitis, brainstem encephalitis and encephalomyelitis (including AFP). Although this implies that stages 2,3 and 4 are severe, ICU admission is only recommended for stages 3 and stage 4.

All three schemes acknowledge that there is a continuum of severity from uncomplicated HFMD through to fatal cardiopulmonary failure [33, 255, 268].

Overall, the WHO, Vietnamese and Chinese guidelines all focus on early supportive care with careful observation to recognize disease progression as the cornerstone of case management. IVIG is recommended in all three guidelines to treat cases with severe neurological involvement such as cranial nerve palsy, nystagmus, flaccid paralysis, and those with cardiopulmonary complications.

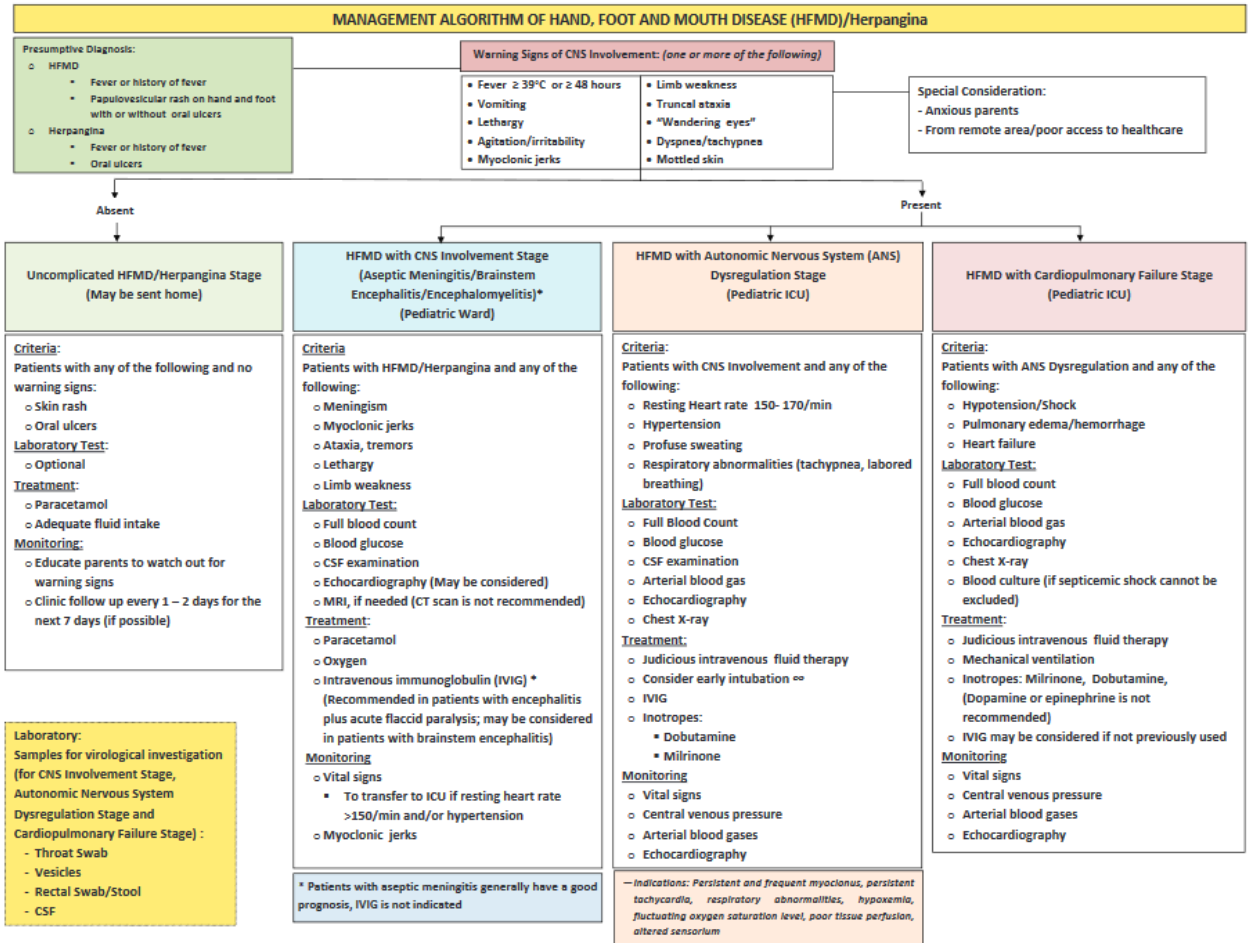
Table 5.2: Vietnam stage based management system for HFMD.

Table 1.4 Vietnam stage based management system[86, 184]

Grade 1	Grade 2a	Grade 2b[a]	Grade 3*	Grade 4*
Rash with or without ulcers in the mouth	History from carers of myoclonus > 2times in 30minutes	Ataxia*	HR>170beats/min (afebrile and at rest)	Apnoea
Ulcers in the mouth	Irritability	Nystagmus*	Peripherally cold	SpO2<92%
Fever		Weak limbs (<4/5)*	Systolic Blood pressure • <1year >110mmHg • 1-2 years >115mmHg • >2 years >120mmHg	Shock: Undetectable BP
		Cranial nerve paralysis*	Raised respiratory rate	Low systolic BP: <12 mths: <70mmHg >12 mths: <80mmHg
		Myoclonus witnessed by clinician > 2times in 30minutes	GCS<10	Pulse pressure (systolic – diastolic)<25mmHg
		Lethargy	Irregular breathing pattern	
		HR>150 beats/min (afebrile and at rest)	Abdominal respiration	
			Temp>39, with antipyretics	

[a]Indication for admission to paediatric intensive care (PICU) *Indications for intravenous immunoglobulin (IVIG). HR: Heart Rate, Temp: Temperature, GCS: Glasgow Coma Scale

Table 5.3: World Health Organisation guide to clinical management and public health response for HFMD.



5.8.3 Usability of WHO, Chinese and Vietnamese schemes

When trying to apply the three schemes to the cohort dataset, a number of issues with usability were identified.

1. A range of clinical indicators are listed but there is no specification as to whether any, all or a specified number of indicators must be present for the patient to be placed in that class. This is likely to result in the over-classification of cases as severe e.g. a child with isolated tachycardia could be placed in Chinese level 4. In clinical case classification schemes used for other diseases, such as dengue, much more clarity about the application of clinical indicators is achieved by using terms such as "*Fever and two of the following*", "*with at least one of the following criteria*", "*any of the following*".

2. In the Chinese scheme, the classes are not mutually exclusive as the presence of a particular clinical indicators, such as tachycardia and tachypnoea, could put a patient in two different classes.

3. Some clinical indicators do not seem logical e.g. a GCS <10 places a patient in grade 3 respiratory and circulatory failure in the Vietnamese system, whereas this might be attributable to CNS involvement without respiratory and circulatory complications.

4. Some non-specific clinical indicators such as vomiting (Vietnamese) and lethargy (Vietnamese, Chinese and WHO) are used to indicate CNS complications. This may lead to over-classification of cases as having CNS complications.

5. The WHO scheme includes warning signs, but it is not clear how these warning signs should be used to classify patients. There are algorithmic lines that indicate wawrning signs as 'absent' in uncomplicated, but 'present' in the

other stages. However, there are patients with only rash plus warning signs, and it is not clear into which category they should be placed.

6. In the WHO scheme, the classes have a dependency, where moving into a higher severity class requires first meeting the criteria to place you in the lower class. For example, “HFMD with ANS involvement requires”, “Patients with CNS involvement and any of the following”. This presents difficulties when trying to place a patient in a class when they do not meet the criteria for the preceding class e.g. a patient with ANS dysfunction but without CNS signs would not be classifiable if the scheme was strictly followed.

Case classification

6139 inpatients were included in the cohort. Tables 5.4 and 5.5 show the numbers of subjects classified according to the WHO and Vietnam schemes respectively. Few cases were unclassifiable. Table 5.6 shows the Vietnam system collapsed into four categories, to allow more direct comparison with the WHO scheme. A side by side comparison of applying the WHO and Vietnamese schemes to the cohort data is shown in figure 5.1 . It can be seen that the WHO scheme places more patients in grade 2 than the Vietnamese system, and fewer in grade 1.

Table 5.4: Cohort classification using WHO scheme.

WHO scheme						
Composite severity	WHO Uncomplicated	CNS involvement	Early cardiopulmonary failure	Cardiopulmonary failure	Unclassifiable	Total
Non-severe	3359	2203	46	2	31	5841
Severe	18	152	110	14	4	298
Subtotal	3577	2355	156	16	35	6139

Table 5.5: Cohort classification using Vietnamese scheme.

Vietnamese scheme							
Composite severity	Grade1	Grade2a*	Grade2b	Grade3	Grade4	Unclassifiable	Total
Non-severe	4896	360	354	57	158	16	5841
Severe	43	3	103	128	17	4	298
Total	4939	363	457	185	175	20	6139

Table 5.6: Cohort classification using Vietnamese in four classes.

Vietnamese adapted scheme in four classes						
Composite severity	Grade1	Grade2a+b	Grade3	Grade4	Unclassifiable	Total
Non-severe	4986	714	57	158	16	5841
Severe	43	106	128	17	4	298
Subtotal	4939	820	185	175	20	6139

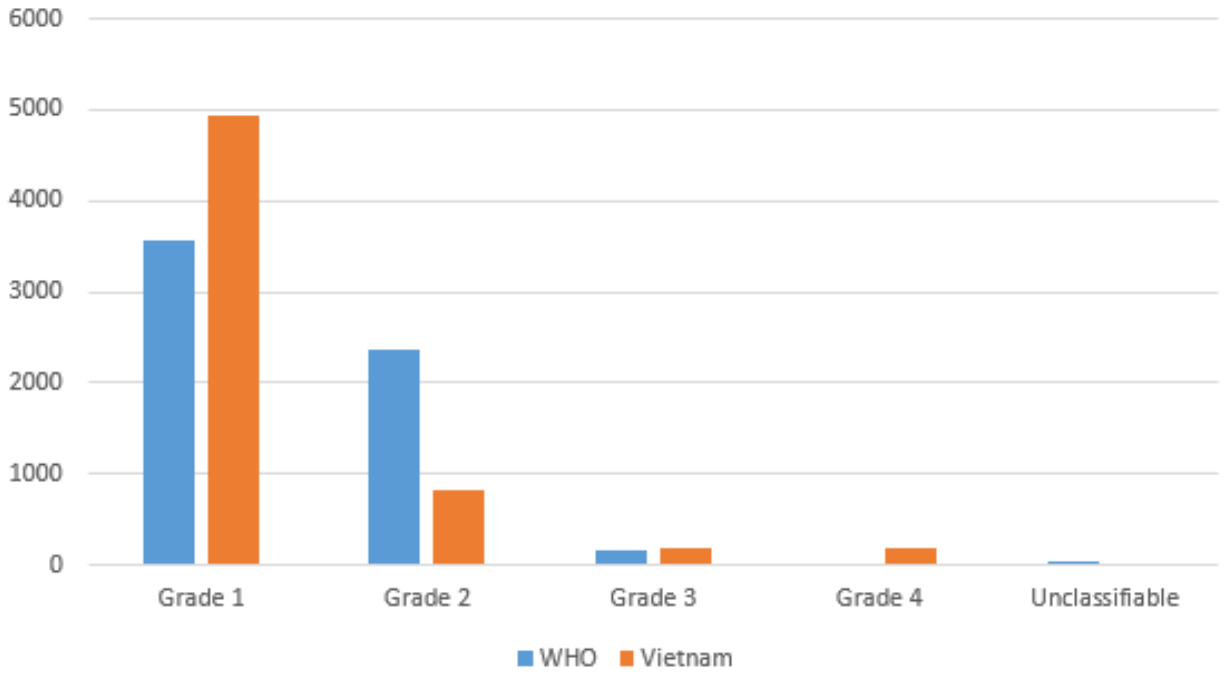


Figure 5.1: Side by side comparison of applying the WHO and Vietnamese schemes to the cohort data.

Concordance of clinical case classification schemes

In comparing the agreement of the WHO scheme to classify severe cases, WHO stages 3 and 4 were classed as severe since these two classes are associated with a recommendation for ICU admission (table 5.3). Table 5.7 shows the concordance between WHO classification of severe and non-severe cases compared to a classification based on actual clinical interventions.

Using the WHO classification scheme, 99% (5762/5810) of the cases were in agreement with non-severe case definition but only 42% (124/294) were in agreement with severe case definition. This corresponds to a specificity of 99% and sensitivity of 42%. The Cohen kappa coefficient was 0.51 (95% CI 0.43- 0.60), representing moderate agreement between the WHO classification and the classification based on actual level of medical intervention. The positive predictive value is 72% (95%CI 65-78%) whilst the negative predictive value was 97% (95%CI 96-97%).

Table 5.7: Concordance between WHO classification and clinical interventions in identifying severe cases of HFMD.

	WHO		
Based on clinical interventions	Non severe	severe	total
Non severe	5762	48	5810
Severe	170	124	294
Total	5932	172	6104

When comparing the agreement of the Vietnam scheme with the clinically defined severe cases, we classified Vietnam stages 2b, 3 and 4 as severe since these three classes are associated with a recommendation for ICU admission (table 5.4). Table 5.8 shows the concordance between the Vietnam classification of severe and non-severe cases compared to a classification based on actual clinical interventions.

Using the Vietnamese system, 90% (5256/5825) of the cases agreed with the non-severe case definition and 84% (248/294) of severe cases. This corresponds to a specificity of 90% (95% CI 89-91%) and sensitivity of 84% (95% CI 80-88%). The Cohen kappa coefficient is 0.40 (95% CI 0.35- 0.46), representing fair agreement between the Vietnam classification and the classification based on actual level of medical intervention. The positive predictive value is however only 30% (95%CI 28-32%) because the WHO system has low specificity and classifies a large number of false positives. The negative predictive value is 99% (95%CI 99-99%).

Table 5.8: Concordance between Vietnam classification and clinical interventions in identifying severe cases of HFMD.

	Vietnam		
Based on clinical interventions	Non severe (1,2a)	severe (2b,3,4)	total
Non severe	5256	569	5825
Severe	46	248	294
Total	5302	817	6119

The WHO and Vietnam systems can be collapsed into four very broadly similar classes: uncomplicated, CNS involvement, and early and late cardiopulmonary/ANS dysregulation. Table 5.9 shows the correlation of the two systems. The Cohen kappa coefficient for the four grades of the WHO and VTN schemes is 0.36 (95% CI 0.33- 0.39), representing fair agreement.

Table 5.9: Correlation of the two classification systems..

	WHO					
Vietnam	Uncomplicated	CNS involvement	Early cardiopulmonary failure	Cardiopulmonary failure	Unclassifiable	Total
1	3400	1520	3	2	14	4939
2a+b	91	712	13	2	2	820
3	4	37	135	9	0	185
4	82	84	5	3	1	175
Unclassifiable	0	2	0	0	18	20
Total	3577	2355	156	16	35	6139

Agreement of existing severity schemes

Using four severity classes, the AUROC for the composite severe case definition for the Vietnam scheme is 0.87 (95%CI 0.84, 0.89) whereas the AUROC for the composite severe case definition for the WHO scheme is 0.85 (95%CI 0.83, 0.87) (figure 5.2). Figure 5.3 shows the AUROC curves when using a binary outcome of non-severe or severe. In this binary analysis the crude AUROC for the Vietnam scheme is 0.73 (95%CI 0.70, 0.76) and for the WHO scheme is 0.71 (95%CI 0.68, 0.74).

Table 10 shows the sensitivity analysis of the agreement of the WHO and Vietnamese schemes using a range of different individual severity indicators, such as mortality, inotrope use etc.

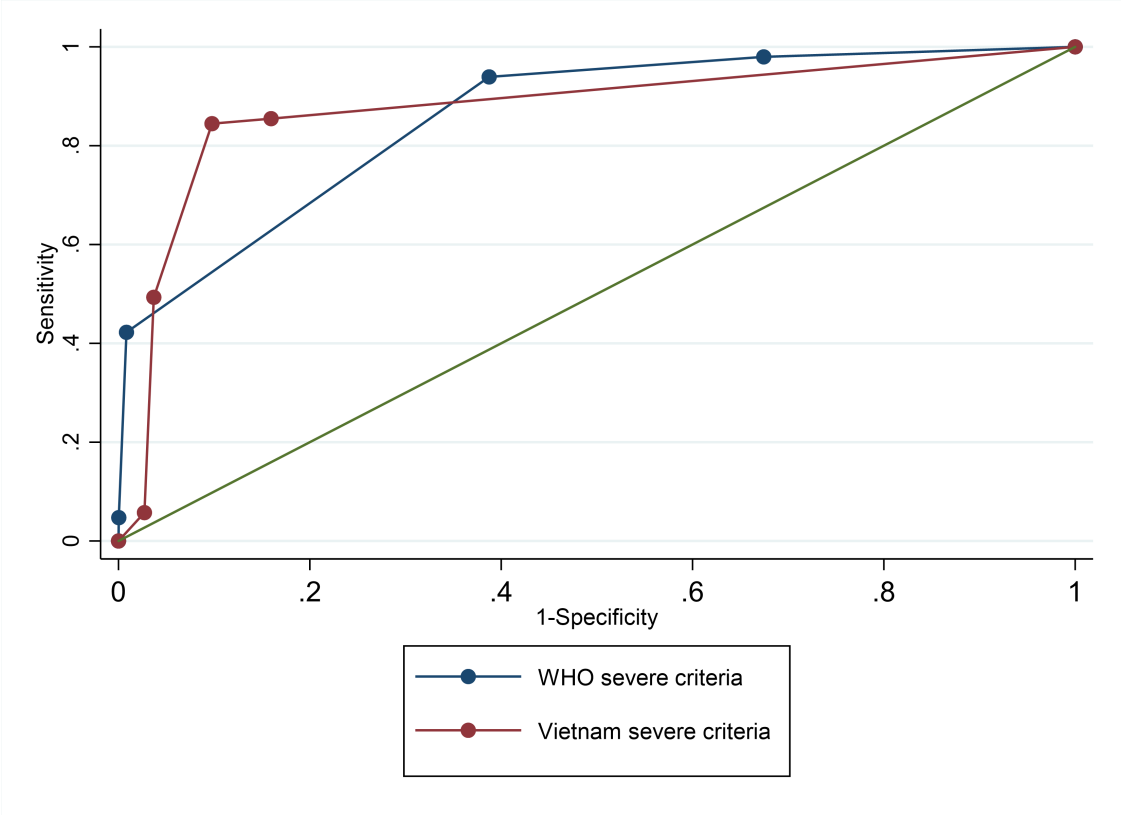


Figure 5.2: Area under the receiver operating characteristic for discriminatory capacity for composite outcome (four classes).

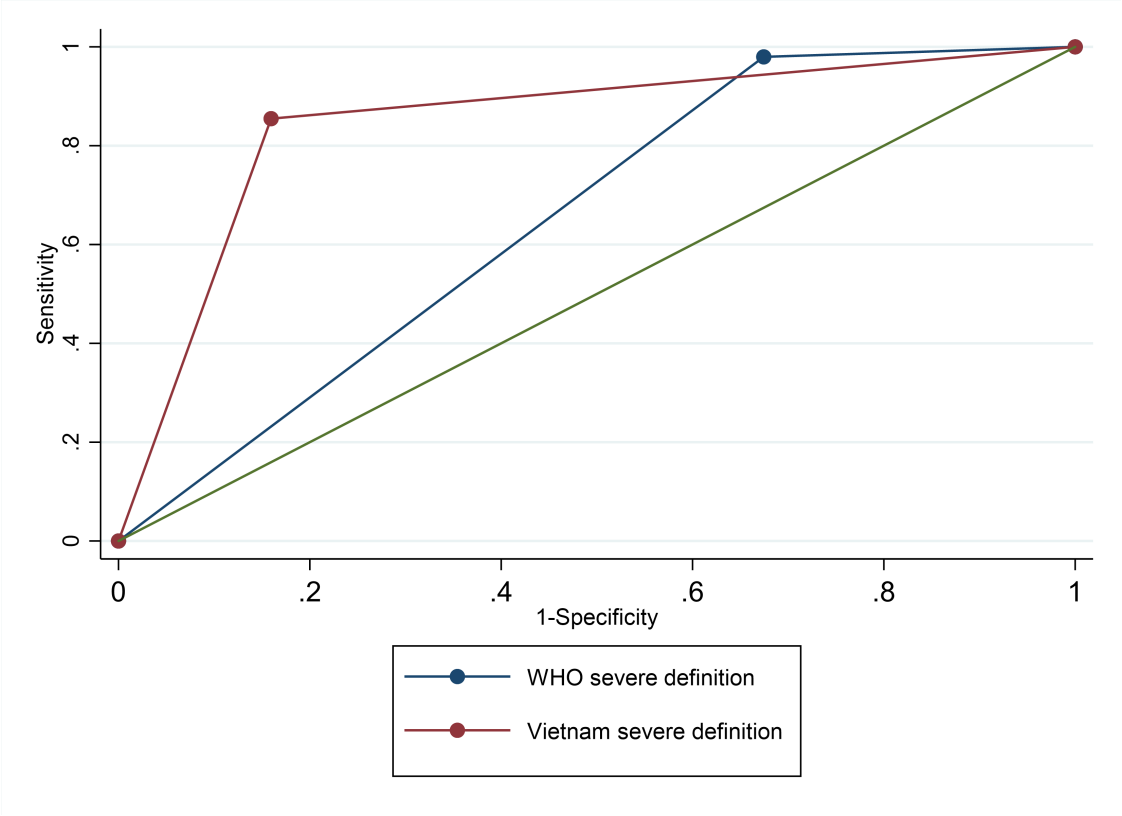


Figure 5.3: Area under the receiver operating characteristic for discriminatory capacity for composite outcome (two classes).

Table 5.10: Sensitivity analyses of WHO and Vietnamese schemes

	WHO	Vietnam
Mortality		
Crude AUROC (95%CI)	0.779 (0, 1)	0.956 (0,1)
Inotrope use		
Crude AUROC (95%CI)	0.887 (0.862, 0.913)	0.896 (0.868, 0.924)
ICU admission		
Crude AUROC (95%CI)	0.861 (0.842, 0.878)	0.877 (0.855, 0.899)
ICU stay ≥ 3days		
Crude AUROC (95%CI)	0.860 (0.841, 0.878)	0.877 (0.855, 0.899)
Ventilation		
Crude AUROC (95%CI)	0.893 (0.864, 0.923)	0.904 (0.873, 0.934)
Ventilation ≥3days		
Crude AUROC (95%CI)	0.887 (0.852, 0.917)	0.896 (0.861, 0.930)
Prolonged stay ≥7 days		
Crude AUROC (95%CI)	0.645 (0.630, 0.661)	0.616 (0.603, 0.630)

5.9 Discussion

The results demonstrated that in an area of mainland China, where HFMD is endemic, the application of the WHO case classification schemes to data from a clinical dataset identified 99% of the non-severe cases, but only 42% of the severe cases, whilst the application of the Vietnamese scheme identified 90% of the non-severe and 84% of the severe cases. Side by side comparison of the two schemes with composite severity demonstrated moderate concordance overall, with particularly discordance in classifying patients with grade 2 severity. Further evaluation based on clinical intervention indicators demonstrated the Vietnamese classification schemes had better agreement in classifying severe cases, with robustness in sensitivity analyses. Despite their broadly similar classes, the comparison of the two schemes showed only fair agreement. A number of issues with usability were also identified; remedying those would improve the utility of these classification schemes.

We have identified a number of challenges in the application of the existing clinical severity case classification schemes for HFMD. There are significant ambiguities in how the schemes and the clinical indicators should be applied. All of the schemes are to some extent ordinal scales, especially the WHO scheme. An ordinal scale is a scale with an intrinsic ranking, going from a milder clinical illness to a more severe one but with no numeric value. For ordinal scales to be effective they need to be unambiguously defined and at any one time a patient should only fall into one of the classes, i.e. the classes must be mutually exclusive. A better ordinal scale for HFMD would allow clear and unambiguous allocation of patients to classes that are an accurate reflection of severity. It would also allow the classification to be used as an outcome measure in clinical trials. Although we did not assess usability in practice or prospectively, we believe that the application of the schemes as written will present difficulties and would likely have limitations in reproducibility.

Comparative analyses showed that the WHO and Vietnamese systems had different performances. The WHO system suffered from poor sensitivity for detecting severe cases, classifying only 42% (124/294) of severe cases. The Vietnamese systems was more sensitive, classifying 84% (248/294) of severe cases, but suffered from poor specificity, misclassifying 569 uncomplicated cases as severe. As a consequence the Vietnamese system had a low positive predictive value. The Vietnamese scheme has a lower kappa score than the WHO scheme, showing lower overall concordance with severity as determined by level of clinical interventions. The two schemes had only moderate concordance with one another.

During epidemic seasons, hospitals would experience significant resource burden such as increased patient volumes, staffing demands, and use of medications or life-supporting equipment. Therefore, early recognition of patients at high risk of poor outcomes is crucial in hospital settings. The WHO scheme had a high specificity of 99%, with a high negative predictive value of 97%. The Vietnam scheme had a specificity of 90% and a high negative predictive value of 99%. This indicated that the schemes may allow the clinician to better discern patients who did not have a severe form of disease caused by NPEVs. The high sensitivity of the case classification system allowed for better case classification and increased admission to healthcare facilities, as evidenced by a low to moderate positive predictive values in both schemes [264]. However, this may overload the healthcare facilities in countries such as China where large numbers of patients are admitted, and close monitoring is required to rule out the risk of developing severe complications. An ideal case classification scheme should have both high sensitivity and specificity to improve recognition in presumptive diagnosis and follow-up of the diseases. Therefore, validation of severity predictors or selection of a highly sensitive test would be warranted in prospective studies in different settings.

In terms of epidemiological surveillance, such case classification schemes could act as standard clinical case definitions that are useful for identifying patients

with severe forms of the disease. For example, neurological complications are common in NPEV infections. A case definition for suspected CNS infection with low sensitivity should be modified by adding additional symptoms or using alternative laboratory criteria; it would then need to be tested rigorously. The broadening of case definition would greatly increase the number of samples from suspected cases with implications in terms of laboratory capacity or costs [269].

This work is, to our knowledge, the first attempt to evaluate the existing HFMD clinical severity classification systems. It was based on one of the largest complete hospitalised HFMD datasets, with a sample size of 6,139 patients over a 2-year period. The data used for this analysis were not primarily collected for such study purposes, therefore, some variables were not entirely consistent with the schemes assessed. However, efforts have been made to generate proxy for these variables and very few patients were unclassifiable.

There are some limitations in this study. First, the revised Chinese case classification scheme was not applied to the data [255]. This was because the evaluation of the revised scheme generated many cases unclassifiable in the preliminary analysis. In practice, the clinicians usually applied the Chinese case classification scheme developed in 2010, with cases without any complication defined as mild cases and those with neurological symptoms or signs or any complication as severe cases [270, 271]. I proposed this chapter based on the first Chinese case classification scheme, but by the time I analysed the data, it was superseded by the revised case classification scheme [255]. In the 2010 Chinese case classification scheme, the definition of mild cases was largely consistent with the definition of uncomplicated cases (HFMD with no complication) in chapter 4. Different from chapter 4, the 2010 scheme grouped the cases with mild complication into severe cases. This scheme has been superseded by a revised scheme developed in 2018, in which the indicators of stages were shortlisted. This led to issues in terms of usability as

mentioned above. There were similar issues on usability, these are summarised in the qualitative evaluation (section 5.8.3).

ICU admission was used as one of the clinical intervention indicators used to assess the agreement of the case classification schemes. However, there is no comprehensive guidance on when patients need critical care admission. Evidence suggested severe outcome was associated with ICU admission in 75% of children with anti-NMDAR encephalitis [272]. Another study using modified Rankin scale reported about half of the patients with acute encephalitis required ICU admission because of coma, seizures or acute respiratory failure [273]. Other severity indicator that has been validated in different diseases, such as mortality, was also used in the sensitivity analyses. The results demonstrated substantial robustness.

In summary, the two schemes evaluated, the WHO and the Vietnamese, both had suboptimal performance. With a severe, life threatening disease in children, sensitivity may be preferred over specificity, in which case the Vietnamese scheme would be preferred. However, this may lead to over-diagnosis of severe disease and, consequently, over treatment. A suggestion of over-treatment was observed in chapter 4. We recommend that the existing HFMD clinical severity case classification schemes are more fully evaluated in prospective studies, as was done for dengue classification schemes, with the aim of developing a more usable scheme that has better operating characteristics than those currently available.

6

Further directions

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6.1 Contribution to the chapter

In this thesis I have presented my work, which aims to improve the characterisation and prioritisation of the global health risks posed by NPEVs and to suggest ways to improve the care of children hospitalised with HFMD.

Chapter 2 describes the detection and reporting of new NPEV types and their geographic distribution. This chapter highlights that there is a significant delay between detection and reporting of new genotypes and that surveillance for, and characterisation of, sub-genotypes associated with more severe illness syndromes is likely to be of greater importance than simply reporting novel genotypes.

Chapter 3 reports the development of a novel enterovirus risk assessment tool, which it is hoped will be of value in identifying, in a transparent and accountable

way, which NEPVs pose the greatest public health risk and therefore warrant attention for surveillance and research. In addition, specific areas for research and development suggested by a panel of experts are presented and can provide the basis for developing a more detailed research agenda for NPEVs.

Chapter 4 provides new large-scale data on children hospitalised with HFMD. It confirms many previous findings, adding weight to the existing evidence, but also challenges other evidence, such as the negative association between the classical rash and severe disease, which I was unable to replicate. More importantly, I have identified some easily and routinely measurable clinical parameters (such as heart rate and blood pressure) that may help in identifying children at risk of severe complications and others, such as myoclonus, which may be leading to over-diagnosis of neurological complications. This chapter also identifies an issue with potential over-treatment with agents such as mannitol and steroids which are of unproven benefit in patients with HFMD. Further work is need to test these hypotheses prospectively.

Chapter 5 presents the first attempt to critically evaluate existing clinical case classification systems for HFMD. I have demonstrated some limitations of these tools and suggest that they require further refinement and evaluation.

6.2 Future directions

Due to their ubiquitous nature, infectivity and high burden, human NPEV will remain a big problem in the foreseeable future. The WHO reported that 3 million children were infected with HFMD in the Asia-Pacific region in 2014, with 90% of these cases occurring in China [87].

Whilst the majority of children will be managed at home, a considerable proportion will need hospital admission. A modelling study estimated that case hospitalisation rate for HFMD was 6% in Asia (95%CI 2.8% to 14.9%) [274]. Even in children with mild HFMD, hospitalisation is a stressful experience for the children and their families [275]. Even more worrying is the fact that these viruses show propensity to affect the central nervous system and to cause severe infections, sequelae and death. In many health systems in low and middle-income countries (LMICs) physiotherapy, ergotherapy and other supportive disciplines, such as neuropsychology are not integrated into the health system [268]. Despite this considerable burden, enterovirus infections do not yet receive the global attention that they deserve, e.g HFMD and NPEVs infections in more general are not listed as causes in the Global Burden of Disease Study 2017 [276].

To prevent these enterovirus-associated diseases, safe and effective vaccines are urgently needed. Two EV-A71 vaccines have been licensed in China (but not internationally), however there is no polyvalent enterovirus vaccine available that would afford protection against the main aetiologies of HFMD (i.e. EV-A71, CV-A16, CV-A10, CV-A6). These vaccines are still very early in their development and have not been tested in clinical trials. Even if a successful candidate was identified, the clinical development could take many years. There is also no vaccine available against EV-D68, a virus that caused outbreaks of respiratory illness and AFP in Europe and the US since 2014. I hope that with my work described in this thesis, I have contributed towards highlighting and assessing the potential threat of newly emerging and re-emerging enteroviruses. I am planning to work and engage with virology societies to seek successful uptake and implementation of the ERAT tool. In addition, I am describing one of the largest clinical observational studies in children with HFMD in China. Whilst there was low mortality, in the severe group $>$ 60% of children needed ICU admission and 25% needed ventilation. It is therefore likely, that when similar HFMD outbreaks would occur in Asian-Pacific

regions with less robust healthcare systems, mortality would be higher, underscoring the urgent need for effective vaccines.

The clinical study also describes the use of supportive therapies for HFMD, e.g. mannitol was used in 47% of children. An evaluation of the benefit of these supportive therapies would be warranted. Finally, I performed a critical review and comparison of three different case classification systems for HFMD and I make a case for improvement and harmonisation of these classification systems. In addition to publishing this work, I am planning to contact colleagues at WHO and China CDC to make them aware of this evaluation.

Further studies could be performed to improve prevention and treatment of NPEV infections. For example, neurological complications associated with NPEV infections can be controlled by vaccination [277]. The implementation of control programmes requires accurate data on disease burden, which is in turn dependent on disease surveillance and standardised case definitions. One limitation of the single centre study presented in chapter 4 is the risk of detection and referral bias in children aged less than 3. Therefore, prospective multi-centre observational studies are appropriate research design to answer these questions. The primary advantage of conducting a multi-centre prospective study is to have a more representative sample of subjects. The planning of multi-centre research could be long and arduous that requires substantial preparation time. Several methodology steps should be warranted. One of the steps involves formulating and refining a research question and conducting pilot studies. Performing systematic review and meta-analysis would help to comprehend the current knowledge and refine the research question, whilst smaller-scale studies could be undertaken to augment the conduct of future studies in a stepwise fashion. Ideally, the research question would lead to a hypothesis. A planning phase involves the acquisition of funding to support the coordination and preparation of the research [278].

There is a need for an efficacious, safe and affordable oral treatment for NPEV infections. Typically, clinical trials in NPEV infections have been small and at an early stage of the development pipeline [277, 279]. Only a few included children, although this is the age group predominantly affected by the disease. Therefore, optimal treatment for neonates and young children remains poorly studies. However, large trial using a standardised trial design with adequate sample size and sufficient statistical power is often limited by ethical and practical problems [280]. In terms of ethics, opportunities for clinical trial may be limited by over-protective ethic committee that was not fully aware of international ethical norms or underestimation of parents' willingness. Practical issues included sufficient funding to support medium-term follow-up [280]. In addition, evaluation on the effectiveness of antiviral properties is generally deferred until the trials reach phase III [281]. This would mean a delay to the access of children to potentially useful medications. Therefore, more trials are needed, especially in areas of high clinical need. Key considerations on trial design included collecting opportunistic pharmacokinetic samples from children, addressing small sample size, and dealing with negative attitude to participation in trials [281]. The latter may be resolved by offering incentives to encourage enrolment and enhance follow-up, etc [282]. However, investigators need to take care to avoid incentives that may be coercive or unduly influence research participants.

Appendices

A

Appendices

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A.1 Appendix to chapter 3

A.1.1 Final round Delphi survey for tool amendment

Section A: Page 1. Disease Severity

The non-polio Enterovirus Risk Assessment Tool (ERAT) is intended as a tool for assessing the relative pandemic/epidemic risk of non-polio enteroviruses. The risk assessment process involves assessment of seven risk elements (attributes that may reflect the risk), where a level of risk is assigned to selected non-polio enteroviruses using the scoring criteria for the risk elements.

The tick box questions below ask you to assign a level of risk to the short list of non-polio enteroviruses suggested by participants in the second round Delphi survey. To see the study protocol and the finalised risk assessment tool, please click here.

A1.

"Disease severity" is one of the core risk elements. It is defined as "the proportion of persons who develop moderate/severe illnesses following infection".

The scoring criteria (risk level) associated with this element are:

1. Low risk: Causes almost exclusively mild, self-limiting illnesses. Moderate or severe illness requiring hospitalisation very rarely seen (
2. Moderate risk: Moderate or severe illness requiring hospitalisation rarely seen (≥1/10,000 and3. High risk: Moderate or severe illness requiring hospitalisation uncommon but not rare (≥1/1,000 clinical cases are moderate or severe).

Please assign <u>one</u> level of risk to <u>all</u> the short-listed non-polio enteroviruses.

	1) Low risk	2) Moderate risk	3) High risk	Unable to answer
Enterovirus A71 (EV-A71)	☐	☐	☐	☐
Coxsackievirus A16 (CV-A16)	☐	☐	☐	☐
Coxsackievirus A6 (CV-A6)	☐	☐	☐	☐
Enterovirus D68 (EV-D68)	☐	☐	☐	☐
Coxsackievirus A10 (CV-A10)	☐	☐	☐	☐
Echovirus 30 (E-30)	☐	☐	☐	☐
Coxsackievirus B3 (CV-B3)	☐	☐	☐	☐
Coxsackievirus A9 (CV-A9)	☐	☐	☐	☐
Echovirus 11 (E-11)	☐	☐	☐	☐
Coxsackievirus B4 (CV-B4)	☐	☐	☐	☐
Coxsackievirus B5 (CV-B5)	☐	☐	☐	☐
Echovirus 6 (E-6)	☐	☐	☐	☐
Coxsackievirus B1 (CV-B1)	☐	☐	☐	☐
Coxsackievirus A21 (CV-A21)	☐	☐	☐	☐
Echovirus 1 (E-1)	☐	☐	☐	☐
Enterovirus A90 (EV-A90)	☐	☐	☐	☐

A2. **Please indicate how confident you are that there is sufficient data and/or understanding to reliably assign risk to these non-polio enteroviruses using the "disease severity" risk element. Please select <u>one</u> only.**

- ☐ Very confident
- ☐ Somewhat confident
- ☐ Neutral
- ☐ Somewhat unconfident
- ☐ Very unconfident
- ☐ Unable to answer

A3. **<u>What</u> research would be needed to increase your level of confidence in the assignment of risk using the "disease severity" risk element? Please provide your response in the comment box below.**

Section B: Page 2. Incidence and distribution

B1.

"Incidence and distribution" is one of the core risk elements. It is defined as "a measure of disease frequency and spatio-temporal trend associated with non-polio enteroviruses over the past five years".

The scoring criteria associated with this element are:

1. Low risk: Evidence of no significant change in epidemiology (stable incidence of cases, frequency of outbreaks; and geographic distribution) over the past 5 years;
2. Moderate risk: Weak or inconclusive evidence of increasing incidence of cases, frequency of cases, and geographic distribution over the past 5 years;3. High risk: Clear evidence of increasing incidence of cases, frequency of outbreaks, geographic distribution and/or recent emergence as a cause of human disease over the past 5 years.

Please assign <u>one</u> level of risk to <u>all</u> the short-listed non-polio enteroviruses.

	1) Low risk	2) Moderate risk	3) High risk	Unable to answer
Enterovirus A71 (EV-A71)	☐	☐	☐	☐
Coxsackievirus A16 (CV-A16)	☐	☐	☐	☐
Coxsackievirus A6 (CV-A6)	☐	☐	☐	☐
Enterovirus D68 (EV-D68)	☐	☐	☐	☐
Coxsackievirus A10 (CV-A10)	☐	☐	☐	☐
Echovirus 30 (E-30)	☐	☐	☐	☐
Coxsackievirus B3 (CV-B3)	☐	☐	☐	☐
Coxsackievirus A9 (CV-A9)	☐	☐	☐	☐
Echovirus 11 (E-11)	☐	☐	☐	☐
Coxsackievirus B4 (CV-B4)	☐	☐	☐	☐
Coxsackievirus B5 (CV-B5)	☐	☐	☐	☐
Echovirus 6 (E-6)	☐	☐	☐	☐
Coxsackievirus B1 (CV-B1)	☐	☐	☐	☐
Coxsackievirus A21 (CV-A21)	☐	☐	☐	☐
Echovirus 1 (E-1)	☐	☐	☐	☐
Enterovirus A90 (EV-A90)	☐	☐	☐	☐

B2. **Please indicate how confident you are that there is sufficient data and/or understanding to reliably assign risk to these non-polio enteroviruses using the "incidence and distribution" risk element. Please select <u>one</u> only.**

- Very confident ☐
- Somewhat confident ☐
- Neutral ☐
- Somewhat unconfident ☐
- Very unconfident ☐
- Unable to answer ☐

B3. **<u>What</u> research would be needed to increase your level of confidence in the assignment of risk using the "incidence and distribution" risk element? Please provide your response in the comment box below.**

Section C: Page 3. Stage of vaccine development

C1.

"Stage of vaccine development" is one of the core risk elements. It is defined as "the development of a candidate vaccine against non-polio enteroviruses and the availability of immunogenicity, safety and efficacy data".

The scoring criteria (risk level) associated with this element are:

1. Low risk: Vaccine available or candidate vaccine with demonstrated safety, immunogenicity, and efficacy from at least one phase III trials;
2. Moderate risk: Candidate vaccine with safety and immunogenicity data from phase II or observational studies but no phase III trial data;3. High risk: No candidate vaccine or a candidate vaccine with only preclinical or phase I data.

Please assign <u>one</u> level of risk to <u>all</u> the short-listed non-polio enteroviruses.

	1) Low risk	2) Moderate risk	3) High risk	Unable to answer
Enterovirus A71 (EV-A71)	☐	☐	☐	☐
Coxsackievirus A16 (CV-A16)	☐	☐	☐	☐
Coxsackievirus A6 (CV-A6)	☐	☐	☐	☐
Enterovirus D68 (EV-D68)	☐	☐	☐	☐
Coxsackievirus A10 (CV-A10)	☐	☐	☐	☐
Echovirus 30 (E-30)	☐	☐	☐	☐
Coxsackievirus B3 (CV-B3)	☐	☐	☐	☐
Coxsackievirus A9 (CV-A9)	☐	☐	☐	☐
Echovirus 11 (E-11)	☐	☐	☐	☐
Coxsackievirus B4 (CV-B4)	☐	☐	☐	☐
Coxsackievirus B5 (CV-B5)	☐	☐	☐	☐
Echovirus 6 (E-6)	☐	☐	☐	☐
Coxsackievirus B1 (CV-B1)	☐	☐	☐	☐
Coxsackievirus A21 (CV-A21)	☐	☐	☐	☐
Echovirus 1 (E-1)	☐	☐	☐	☐
Enterovirus A90 (EV-A90)	☐	☐	☐	☐

C2. **Please indicate how confident you are that there is sufficient data and/or understanding to reliably assign risk to these non-polio enteroviruses by using the "stage of vaccine development" risk element. Please select <u>one</u> only.**

- ☐ Very confident
- ☐ Somewhat confident
- ☐ Neutral
- ☐ Somewhat unconfident
- ☐ Very unconfident
- ☐ Unable to answer

C3. **<u>What</u> research would be needed to increase your level of confidence in the assignment of risk using the "stage of vaccine development" risk element? Please provide your response in the comment box below.**

Section D: Page 4. Stage of antiviral drug development

D1.

"Stage of antiviral drug development" is one of the core risk elements. It is defined as "the development of antiviral drugs and compounds with evidence of safety and non-polio enterovirus efficacy for use in at-risk human populations".

The scoring criteria (risk level) associated with this element are:

1. Low risk: Antiviral with demonstrated safety and efficacy from at least one phase III trial;
2. Moderate risk: Antiviral with safety and efficacy data from phase II or observational studies but no phase III trial data;3. High risk: No antiviral or a candidate antiviral with only preclinical or phase I data.

Please assign <u>one</u> level of risk to <u>all</u> the short-listed non-polio enteroviruses.

	1) Low risk	2) Moderate risk	3) High risk	Unable to answer
Enterovirus A71 (EV-A71)	☐	☐	☐	☐
Coxsackievirus A16 (CV-A16)	☐	☐	☐	☐
Coxsackievirus A6 (CV-A6)	☐	☐	☐	☐
Enterovirus D68 (EV-D68)	☐	☐	☐	☐
Coxsackievirus A10 (CV-A10)	☐	☐	☐	☐
Echovirus 30 (E-30)	☐	☐	☐	☐
Coxsackievirus B3 (CV-B3)	☐	☐	☐	☐
Coxsackievirus A9 (CV-A9)	☐	☐	☐	☐
Echovirus 11 (E-11)	☐	☐	☐	☐
Coxsackievirus B4 (CV-B4)	☐	☐	☐	☐
Coxsackievirus B5 (CV-B5)	☐	☐	☐	☐
Echovirus 6 (E-6)	☐	☐	☐	☐
Coxsackievirus B1 (CV-B1)	☐	☐	☐	☐
Coxsackievirus A21 (CV-A21)	☐	☐	☐	☐
Echovirus 1 (E-1)	☐	☐	☐	☐
Enterovirus A90 (EV-A90)	☐	☐	☐	☐

D2. **Please indicate how confident you are that there is sufficient data and/or understanding to reliably assign risk to these non-polio enteroviruses by using the "stage of antiviral drug development" risk element. Please select <u>one</u> only.**

- ☐ Very confident
- ☐ Somewhat confident
- ☐ Neutral
- ☐ Somewhat unconfident
- ☐ Very unconfident
- ☐ Unable to answer

D3. **<u>What</u> research would be needed to increase your level of confidence in the assignment of risk by using the "stage of antiviral drug development" risk element? Please provide your response in the comment box below.**

Section E: Page 5. Genetic diversity

E1.

"Genetic diversity" is one of the supplementary risk elements. It is defined as "the diversity of phylogenetically distinct variants and/or the rate of nucleotide substitutions and/or recombination events".

The scoring criteria (risk level) associated with this element are:

-1. Low risk: Stable genetic diversity of circulating variants (over the past 5 years);
0. Unknown risk: No data or insufficient data;+1. High risk: Increasing genetic diversity of circulating variants (over the past 5 years).

Please assign <u>one</u> level of risk to <u>all</u> the short-listed non-polio enteroviruses.

** Please choose "unable to answer" where appropriate if you do not know enough about the availability of data as opposed to knowing there is no or insufficient data ("unknown risk").*

	-1) Low risk	0) Unknown risk	+1) High risk	Unable to answer*
Enterovirus A71 (EV-A71)	☐	☐	☐	☐
Coxsackievirus A16 (CV-A16)	☐	☐	☐	☐
Coxsackievirus A6 (CV-A6)	☐	☐	☐	☐
Enterovirus D68 (EV-D68)	☐	☐	☐	☐
Coxsackievirus A10 (CV-A10)	☐	☐	☐	☐
Echovirus 30 (E-30)	☐	☐	☐	☐
Coxsackievirus B3 (CV-B3)	☐	☐	☐	☐
Coxsackievirus A9 (CV-A9)	☐	☐	☐	☐
Echovirus 11 (E-11)	☐	☐	☐	☐
Coxsackievirus B4 (CV-B4)	☐	☐	☐	☐
Coxsackievirus B5 (CV-B5)	☐	☐	☐	☐
Echovirus 6 (E-6)	☐	☐	☐	☐
Coxsackievirus B1 (CV-B1)	☐	☐	☐	☐
Coxsackievirus A21 (CV-A21)	☐	☐	☐	☐
Echovirus 1 (E-1)	☐	☐	☐	☐
Enterovirus A90 (EV-A90)	☐	☐	☐	☐

E2. **Please indicate how confident you are that there is sufficient data and/or understanding to reliably assign risk to these non-polio enteroviruses by using the "genetic diversity" risk element. Please select <u>one</u> only.**

- ☐ Very confident
- ☐ Somewhat confident
- ☐ Neutral
- ☐ Somewhat unconfident
- ☐ Very unconfident
- ☐ Unable to answer

E3. **<u>What</u> research would be needed to increase your level of confidence in the assignment of risk by using the "genetic diversity" risk element? Please provide your response in the comment box below.**

Section F: Page 6. Tissue Tropism

F1.

"Tissue tropism" is one of the supplementary risk elements. It is defined as "the type of tissues and cells that support growth of the virus".

The scoring criteria (risk level) associated with this element are:

-1. Low risk: Data indicate a lack of tropism for human respiratory, cardiac, neurological and/or renal tissue cells;
0. Unknown risk: No data or insufficient data;+1. High risk: Data indicate tropism for human respiratory, cardiac, neurological, and/or renal tissue cells.

Please assign <u>one</u> level of risk to <u>all</u> the short-listed non-polio enteroviruses.

** Please choose "unable to answer" where appropriate if you do not know enough about the availability of data as opposed to knowing there is no or insufficient data ("unknown risk").*

	-1) Low risk	0) Unknown risk	+1) High risk	Unable to answer*
Enterovirus A71 (EV-A71)	☐	☐	☐	☐
Coxsackievirus A16 (CV-A16)	☐	☐	☐	☐
Coxsackievirus A6 (CV-A6)	☐	☐	☐	☐
Enterovirus D68 (EV-D68)	☐	☐	☐	☐
Coxsackievirus A10 (CV-A10)	☐	☐	☐	☐
Echovirus 30 (E-30)	☐	☐	☐	☐
Coxsackievirus B3 (CV-B3)	☐	☐	☐	☐
Coxsackievirus A9 (CV-A9)	☐	☐	☐	☐
Echovirus 11 (E-11)	☐	☐	☐	☐
Coxsackievirus B4 (CV-B4)	☐	☐	☐	☐
Coxsackievirus B5 (CV-B5)	☐	☐	☐	☐
Echovirus 6 (E-6)	☐	☐	☐	☐
Coxsackievirus B1 (CV-B1)	☐	☐	☐	☐
Coxsackievirus A21 (CV-A21)	☐	☐	☐	☐
Echovirus 1 (E-1)	☐	☐	☐	☐
Enterovirus A90 (EV-A90)	☐	☐	☐	☐

F2. **Please indicate how confident you are that there is sufficient data and/or understanding to reliably assign risk to these non-polio enteroviruses by using the "tissue tropism" risk element. Please select <u>one</u> only.**

- ☐ Very confident
- ☐ Somewhat confident
- ☐ Neutral
- ☐ Somewhat unconfident
- ☐ Very unconfident
- ☐ Unable to answer

F3. **<u>What</u> research would be needed to increase your level of confidence in the assignment of risk by using the "tissue tropism" risk element? Please provide your response in the comment box below.**

Section G: Page 7. Population susceptibility

G1.

"Population susceptibility" is one of the supplementary risk elements. It is defined as "the extent of direct or cross-protective immunity to specific non-polio enterovirus genotype/serotypes in the human populations".

The scoring criteria (risk level) associated with this element are:

-1. Low risk: Likely to be widespread (>50%) direct or cross-protective immunity in children under 15 years of age;
0. Unknown risk: No data or insufficient data;+1. High risk: Likely to be widespread (>50%) susceptibility to infection in children under 15 years of age.

Please assign <u>one</u> level of risk to <u>all</u> the short-listed non-polio enteroviruses.

** Please choose "unable to answer" where appropriate if you do not know enough about the availability of data as opposed to knowing there is no or insufficient data ("unknown risk").*

	-1) Low risk	0) Unknown risk	+1) High risk	Unable to answer*
Enterovirus A71 (EV-A71)	☐	☐	☐	☐
Coxsackievirus A16 (CV-A16)	☐	☐	☐	☐
Coxsackievirus A6 (CV-A6)	☐	☐	☐	☐
Enterovirus D68 (EV-D68)	☐	☐	☐	☐
Coxsackievirus A10 (CV-A10)	☐	☐	☐	☐
Echovirus 30 (E-30)	☐	☐	☐	☐
Coxsackievirus B3 (CV-B3)	☐	☐	☐	☐
Coxsackievirus A9 (CV-A9)	☐	☐	☐	☐
Echovirus 11 (E-11)	☐	☐	☐	☐
Coxsackievirus B4 (CV-B4)	☐	☐	☐	☐
Coxsackievirus B5 (CV-B5)	☐	☐	☐	☐
Echovirus 6 (E-6)	☐	☐	☐	☐
Coxsackievirus B1 (CV-B1)	☐	☐	☐	☐
Coxsackievirus A21 (CV-A21)	☐	☐	☐	☐
Echovirus 1 (E-1)	☐	☐	☐	☐
Enterovirus A90 (EV-A90)	☐	☐	☐	☐

G2. **Please indicate how confident you are that there is sufficient data and/or understanding to reliably assign risk to these non-polio enteroviruses by using the "population susceptibility" risk element? Please select <u>one</u> only.**

- ☐ Very confident
- ☐ Somewhat confident
- ☐ Neutral
- ☐ Somewhat unconfident
- ☐ Very unconfident
- ☐ Unable to answer

G3. **<u>What</u> research would be needed to increase your level of confidence in the assignment of risk by using the "pouplation susceptibility" risk element? Please provide your response in the comment box below.**

Section H: Page 8. Ranking the risk elements according to the importance

In this section, you will be asked to rank all the risk elements according to their relative importance. The ranked order will be used to approximate the weight of each risk element, which express the desired contribution to the final score for risk of non-polio enteroviruses.

H1. **How would you rank <u>all</u> the risk elements from most important to least important? <u>Please state the most important element on top of the list and the least important one at the bottom of the list</u>.**

- Disease severity ☐
- Incidence and distribution ☐

Stage of vaccine development

Stage of antiviral drug development

Genetic diversity

Tissue tropism

Population susceptibility

Section I: Page 9. Basic information

I1. Which of the following category best describes your expertise? Please check <u>all</u> that apply or state in the text box.

This information will be anonymised during data analyses and compiled as a group in the report.

- Clinical Medicine
- Virology
- Epidemiology
- Immunology
- Geospatial science
- Pharmacology
- Vaccinology
- Bioinformatics
- Other

Other

I2. Which of the following best describes the geographical location of your research or expertise? Please check <u>all</u> only.

- Americas
- Europe
- Asia and Australia
- Africa and Middle East

I3.

Please provide your title, name and institutional affiliation so that we can give feedback to you. Please state your information in the text box.

Title

The information provided will be used to compile a list of participants and shown in the final report only. Your responses will not be identified by individual. All information above will be kept confidential and anonymised during data analyses.

I4.

Please provide your title, name and institutional affiliation so that we can give feedback to you. Please state your information in the text box.

Full name

The information provided will be used to compile a list of participants and shown in the final report only. Your responses will not be identified by individual. All information above will be kept confidential and anonymised during data analyses.

I5.

Please provide your title, name and institutional affiliation so that we can give feedback to you. Please state your information in the text box.

Institutional affiliation

The information provided will be used to compile a list of participants and shown in the final report only. Your responses will not be identified by individual. All information above will be kept confidential and anonymised during data analyses.

Thank you for your time spent taking this survey. Your response has been recorded.

We will analyse and publish the results soon.

A.2 Appendix to chapter 4

Table A.1: Primers and probes used in the clinical specimen testing

Target gene	Primer or probe	Sequence (5'- 3')	Flurorophore
EV-A71 (255bp)	Forward primer	GCCCTCACTCACGCTCTAC	VIC-BHQ1
	Reverse primer	TGGGTTAGTTGTGCCCTyAAG	
	Probe primer	TCTCTCCAACTAATCCCGCCCTACTGA	
CV-A16	Forward primer	AACCATCACTCCACACAGGAG	ROX-BHQ1
	Reverse primer	GTACCTGTGGTGGGCATTG	
	Probe primer	CAGCCATTGGGAATTTCTTTAGCCG	
Pan-enterovirus (147 bp)	Forward primer	CCCTGAATGCGGCTAATCC	FAM-BHQ1
	Reverse primer	ATTGTCACCATAACGAGCCA	
	Probe primer	AACCGACTACTTTGGGTGTCCGTGTTTC	
IAC (149 bp)	Forward primer	CACTCGTGAGCACGTATC	CY5-BHQ2
	Reverse primer	TGAGATGCACCATAGCTCAC	
	Probe primer	GAGACCTCTGACTGCCCAACGAG	

A.3 Appendix to chapter 5

WHO severity classification scheme and stage-based management (adapted from version 2011)

Patients have one of the following signs should be categorised as

Table A.2

Uncomplicated HFMD [1]	**HFMD with CNS involvement** [2, 3]	**Early cardiopulmonary failure** [3]	**Cardiopulmonary failure** [3]
Patient with any of the following and no warning sign:	Patient with HFMD and any of the following	Heart rate 150-170 beats/min (at rest)	Hypotension or shock
Skin rash	Meningism	Hypertension	Pulmonary oedema/haemorrhage
Oral ulcers	Myoclonus	Profuse sweating	Heart failure
	Ataxia	Respiratory abnormalities (tachypnea, laboured breathing)	
	Tremor		
	Lethargy		
	Limb weakness		
Stage-based treatment			
Stage 1	Stage 2	Stage 3	Stage 4
May be outpatient	Hospitalisation	ICU admission	ICU admission
Monitor for warning signs	Monitor vital signs	Invasive monitoring for vital signs, arterial blood gas and echocardiography	Closely monitor vital signs, invasive monitoring if feasible
Paracetamol	Paracetamol	Early intubation	Mechanical ventilation
Fluid intake	Oxygen therapy	Judicious intravenous fluid therapy	Judicious intravenous fluid therapy
Follow-up examination	Supportive treatment	Vasoactive agents (milrinone, dobutamine)	Vasoactive agents (dopamine or epinephrine is not recommended)
		IVIg use	IVIg use (may be considered)
	IVIg use *		

[2] warning signs include fever>=39, fever>=2days, vomiting, lethargy, agitation/irritability, myoclonus, limb weakness, truncal ataxia, nystagmus, dyspnoea, tachypnea, mottled skin.

[3] a severe case definition is not given. But severe forms of complications include aseptic meningitis, brainstem encephalitis, or encephalitis.

* IVIg is not indicated for patient with aseptic meningitis, as the prognosis is good.

Vietnamese severity classification scheme (adapted from version 2012, Saba2015, Qui2016)

Patients have one of the following signs should be categorised as

Table A.3

Grade 1 uncomplicated HFMD	**Grade 2a CNS complications**	**Grade 2b [1] Severe CNS complication**	**Grade 3 [1] Respiratory and circulatory failure**	**Grade 4 [1] Severe respiratory and circulatory failure**
Rash with/without ulcers in the mouth	History from carer of myoclonus <2times/30 minutes	Ataxia (limb/body shaking, unsteady sitting/gait) *	HR>170 beats/min (afebrile and at rest)	Apnoea
Ulcers in the mouth	Lethargy, agitation, irritability	Nystagmus *	Profuse sweating or peripherally cold	Livid skin/ SPO2<92%
	Fever ≥39℃ or fever>2days	Weak limb (<4/5) or acute flaccid paralysis *	Systolic BP increases with age <1y: >110mmHg; 1-2y: >115mmHg; >2y: >120mmHg	Acute pulmonary oedema
	Vomiting	Cranial nerve palsy *	Increased respiratory rate	Shock with undetectable BP
		Myoclonus witnessed by clinician ≥2times/30mintues	Coma or GCS<10	Low systolic BP: <12mo: <70mmHg, >12mo: <80mmHg
		Lethargy	Irregular breathing pattern	Pulse pressure≤25mmHg
		HR>150 beats/min (afebrile and at rest)	Abdominal respiration	
		Temp≥39℃, with antipyretics		
Stage-based treatment				
Grade1	Grade 2a	Grade 2b	Grade 3	Grade 4
Outpatient	Hospitalisation	ICU admission	ICU admission	ICU admission
Monitoring severe signs	Monitor vital signs	Monitor vital signs	Monitor vital signs	Closely monitor vital signs, invasive BP monitoring
Daily follow-up monitoring until day 8	Oral phenobarbital	IV phenobarbital	IV phenobarbital	IV phenobarbital
		IVIg use when indicated *	IVIg use	IVIg use
		Antibiotic if purulent meningitis is not ruled out	Antibiotic if purulent meningitis is not ruled out	Antibiotic if purulent meningitis is not ruled out
			Vasoactive agents (milrinone, dobutamine)	Vasoactive agents
			Oxygen therapy	Intubation or ventilation
			Parenteral nutrition	Treat complications

[1] Grade 2b, 3 and 4 is defined as severe HFMD.

* Indication for the use of IVIg.

References

1. Huvos, A. & Khan, W. A new paradigm in global health security. *Eastern Mediterranean Health Journal* **22** (2016).

2. *Genus: Enterovirus* (International Committee on Taxomomy of Viruses). https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/picornavirales/w/picornaviridae/681/genus-enterovirus. Accessed 25 Jul 2019.

3. Picornaviridae.com. *Enteroviruses* http://www.picornaviridae.com/enterovirus/enterovirus.htm. Accessed 5 May 2020.

4. Fields, B., Knipe, D. & Howley, P. *Fields virology, Wolters Kluwer* 2013.

5. Pons-Salort, M. & Grassly, N. C. Serotype-specific immunity explains the incidence of diseases caused by human enteroviruses. *Science* **361,** 800–803 (2018).

6. Melnick, J. L. The discovery of the enteroviruses and the classification of poliovirus among them. *Biologicals* **21,** 305–309 (1993).

7. Suresh, S., Forgie, S. & Robinson, J. Non-polio Enterovirus detection with acute flaccid paralysis: A systematic review. *Journal of medical virology* **90,** 3–7 (2018).

8. Leitch, E. M. *et al.* Transmission networks and population turnover of echovirus 30. *Journal of Virology* **83,** 2109–2118 (2009).

9. Phan, T. Q. *Severe Hand Foot and Mouth Disease in Vietnamese Children: Clinical Features and Management Strategies* PhD thesis (The Open University, 2018).

10. Santti, J., Hyypiä, T., Kinnunen, L. & Salminen, M. Evidence of recombination among enteroviruses. *Journal of Virology* **73,** 8741–8749 (1999).

11. Caro, V., Guillot, S., Delpeyroux, F. & Crainic, R. Molecular strategy for 'serotyping' of human enteroviruses. *Journal of General Virology* **82,** 79–91 (2001).

12. Lugo, D. & Krogstad, P. Enteroviruses in the early 21st century: new manifestations and challenges. *Current Opinion in Pediatrics* **28,** 107 (2016).

13. *Enterovirus surveillance guidelines: Guidelines for enterovirus surveillance in support of the Polio Eradication Initiative* (World Health Organisation, 2015). http://www.euro.who.int/___data/assets/pdf_file/0020/272810/EnterovirusSurveillanceGuidelines.pdf.

14. Harvala, H. *et al.* Recommendations for enterovirus diagnostics and characterisation within and beyond Europe. *Journal of clinical virology* **101,** 11–17 (2018).

15. Murphy, F. A. *et al. Virus taxonomy: classification and nomenclature of viruses* (Springer Science & Business Media, 2012).

16. Nikonov, O., Chernykh, E., Garber, M. & Nikonova, E. Y. Enteroviruses: Classification, diseases they cause, and approaches to development of antiviral drugs. *Biochemistry (Moscow)* **82,** 1615–1631 (2017).

17. Beld, M. *et al.* Highly sensitive assay for detection of enterovirus in clinical specimens by reverse transcription-PCR with an armored RNA internal control. *Journal of clinical microbiology* **42,** 3059–3064 (2004).

18. Puenpa, J., Suwannakarn, K., Chansaenroj, J., Vongpunsawad, S. & Poovorawan, Y. Development of single-step multiplex real-time RT-PCR assays for rapid diagnosis of enterovirus 71, coxsackievirus A6, and A16 in patients with hand, foot, and mouth disease. *Journal of Virological Methods* **248,** 92–99 (2017).

19. Nix, W. A., Oberste, M. S. & Pallansch, M. A. Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens. *Journal of clinical microbiology* **44,** 2698–2704 (2006).

20. Tran, C. B. N. *et al.* The seroprevalence and seroincidence of enterovirus71 infection in infants and children in Ho Chi Minh City, Viet Nam. *PloS one* **6,** e21116 (2011).

21. Karelehto, E. *et al.* Enterovirus D68 serosurvey: evidence for endemic circulation in the Netherlands, 2006 to 2016. *Eurosurveillance* **24** (2019).

22. Tsai, J.-D. *et al.* Comparison of the detection rates of RT-PCR and virus culture using a combination of specimens from multiple sites for enterovirus-associated encephalomyelitis during enterovirus 71 epidemic. *Japanese journal of infectious diseases* **67,** 333–338 (2014).

23. Gao, L. *et al.* Spectrum of enterovirus serotypes causing uncomplicated hand, foot, and mouth disease and enteroviral diagnostic yield of different clinical samples. *Clinical Infectious Diseases* **67,** 1729–1735 (2018).

24. Leveque, N. & Laurent, A. A novel mode of transmission for human enterovirus infection is swimming in contaminated seawater: implications in public health and in epidemiological surveillance. *Clinical Infectious Diseases* **47,** 624–626 (2008).

25. Tang, J. W. & Holmes, C. W. Acute and chronic disease caused by enteroviruses. *Virulence* **8,** 1062–1065 (2017).

26. Khetsuriani, N. *et al.* Enterovirus surveillance—United States, 1970–2005. *MMWR Surveill Summ* **55,** 1–20 (2006).

27. Lafolie, J. *et al.* Assessment of blood enterovirus PCR testing in paediatric populations with fever without source, sepsis-like disease, or suspected meningitis: a prospective, multicentre, observational cohort study. *The Lancet Infectious Diseases* **18,** 1385–1396 (2018).

28. *Non-Polio Enterovirus* https://www.cdc.gov/non-polio-enterovirus/index.html.

29. Zhang, X. *et al.* Temporal and long-term trend analysis of class C notifiable diseases in China from 2009 to 2014. *BMJ Open* **6,** e011038 (2016).

30. Pons-Salort, M., Parker, E. P. & Grassly, N. C. The epidemiology of non-polio enteroviruses: recent advances and outstanding questions. *Current opinion in infectious diseases* **28,** 479–487 (2015).

31. Verboon-Maciolek, M. *et al.* Epidemiological survey of neonatal non-polio enterovirus infection in the Netherlands. *Journal of medical virology* **66,** 241–245 (2002).

32. Xing, W. *et al.* Hand, foot, and mouth disease in China, 2008–12: an epidemiological study. *The Lancet infectious diseases* **14,** 308–318 (2014).

33. *A guide to clinical management and public health response for hand, foot and mouth disease (HFMD)* (World Health Organization, Manila, Philippines, 2011). http://iris.wpro.who.int/bitstream/handle/10665.1/5521/9789290615255_eng.pdf.

34. Lu, C.-Y. *et al.* Incidence and case-fatality rates resulting from the 1998 enterovirus 71 outbreak in Taiwan. *Journal of medical virology* **67,** 217–223 (2002).

35. Pons-Salort, M. *et al.* The seasonality of nonpolio enteroviruses in the United States: patterns and drivers. *Proceedings of the National Academy of Sciences* **115,** 3078–3083 (2018).

36. Fraser, C., Riley, S., Anderson, R. M. & Ferguson, N. M. Factors that make an infectious disease outbreak controllable. *Proceedings of the National Academy of Sciences* **101,** 6146–6151 (2004).

37. Delamater, P. L., Street, E. J., Leslie, T. F., Yang, Y. T. & Jacobsen, K. H. Complexity of the basic reproduction number (R0). *Emerging infectious diseases* **25,** 1 (2019).

38. Takahashi, S. *et al.* Hand, foot, and mouth disease in China: modeling epidemic dynamics of enterovirus serotypes and implications for vaccination. *PLoS Medicine* **13,** e1001958 (2016).

39. Lim, C., Jiang, L., Ma, S., James, L. & Ang, L. Basic reproduction number of coxsackievirus type A6 and A16 and enterovirus 71: estimates from outbreaks of hand, foot and mouth disease in Singapore, a tropical city-state. *Epidemiology & Infection* **144,** 1028–1034 (2016).

40. Ma, E. *et al.* Estimation of the basic reproduction number of enterovirus 71 and coxsackievirus A16 in hand, foot, and mouth disease outbreaks. *The Pediatric infectious disease journal* **30,** 675–679 (2011).

41. Fine, P., Eames, K. & Heymann, D. L. Herd immunity: a rough guide. *Clinical infectious diseases* **52,** 911–916 (2011).

42. Huang, J. *et al.* Epidemiology of recurrent hand, foot and mouth disease, China, 2008–2015. *Emerging Infectious Diseases* **24,** 432 (2018).

43. Chang, L.-Y. *et al.* Transmission and clinical features of enterovirus 71 infections in household contacts in Taiwan. *JAMA* **291,** 222–227 (2004).

44. Takahashi, S. *et al.* Hand, foot, and mouth disease in China: modeling epidemic dynamics of enterovirus serotypes and implications for vaccination. *PLoS medicine* **13,** e1001958 (2016).

45. Blanter, M., Sork, H., Tuomela, S. & Flodström-Tullberg, M. Genetic and Environmental Interaction in Type 1 Diabetes: a Relationship Between Genetic Risk Alleles and Molecular Traits of Enterovirus Infection? *Current diabetes reports* **19,** 82 (2019).

46. Ooi, M. H., Wong, S. C., Lewthwaite, P., Cardosa, M. J. & Solomon, T. Clinical features, diagnosis, and management of enterovirus 71. *The Lancet Neurology* **9,** 1097–1105 (2010).

47. Kono, R. *et al.* Pandemic of new type of conjunctivitis. *The Lancet* **299,** 1191–1194 (1972).

48. Tay, C. *et al.* Outbreak of hand, foot and mouth disease in Singapore. *Singapore Med J* **15,** 174–83 (1974).

49. Liu, S.-L. *et al.* Comparative epidemiology and virology of fatal and nonfatal cases of hand, foot and mouth disease in mainland China from 2008 to 2014. *Reviews in medical virology* **25,** 115–128 (2015).

50. Sun, J., Hu, X.-Y. & Yu, X.-F. Current Understanding of Human Enterovirus D68. *Viruses* **11,** 490 (2019).

51. Tokarz, R. *et al.* Worldwide emergence of multiple clades of enterovirus 68. *The Journal of general virology* **93,** 1952 (2012).

52. Holm-Hansen, C. C., Midgley, S. E. & Fischer, T. K. Global emergence of enterovirus D68: a systematic review. *The Lancet Infectious Diseases* **16,** e64–e75 (2016).

53. Dyrdak, R. *et al.* Outbreak of enterovirus D68 of the new B3 lineage in Stockholm, Sweden, August to September 2016. *Eurosurveillance* **21** (2016).

54. Poelman, R., Schölvinck, E. H., Borger, R., Niesters, H. G. & van Leer-Buter, C. The emergence of enterovirus D68 in a Dutch University Medical Center and the necessity for routinely screening for respiratory viruses. *Journal of Clinical Virology* **62,** 1–5 (2015).

55. Böttcher, S. *et al.* Detection of enterovirus D68 in patients hospitalised in three tertiary university hospitals in Germany, 2013 to 2014 (2016).

56. ECDC. *Rapid Risk Assessment – Enterovirus detections associated with severe neurological symptoms in children and adults in European countries ,* (European Centre for Disease Prevention and Control, Stockholm, Sweden, 2016).

57. Chen, N. *et al.* Enterovirus 71 inhibits cellular type I interferon signaling by inhibiting host RIG-I ubiquitination. *Microbial pathogenesis* **100,** 84–89 (2016).

58. Zhang, Y., Li, J. & Li, Q. Immune evasion of enteroviruses under innate immune monitoring. *Frontiers in microbiology* **9** (2018).

59. Tsao, K.-C. *et al.* Responses of IgM for enterovirus 71 infection. *Journal of medical virology* **68,** 574–580 (2002).

60. Vogt, M. R. & Crowe Jr, J. E. Current understanding of humoral immunity to enterovirus D68. *Journal of the Pediatric Infectious Diseases Society* **7,** S49–S53 (2018).

61. Yu, N. *et al.* Evaluation of human enterovirus 71 and coxsackievirus A16 specific immunoglobulin M antibodies for diagnosis of hand-foot-and-mouth disease. *Virology journal* **9,** 1–7 (2012).

62. Lim, H. X. & Poh, C. L. Insights into innate and adaptive immune responses in vaccine development against EV-A71. *Therapeutic Advances in Vaccines and Immunotherapy* **7,** 2515135519888998 (2019).

63. Li, Y. *et al.* Effectiveness of EV-A71 vaccination in prevention of paediatric hand, foot, and mouth disease associated with EV-A71 virus infection requiring hospitalisation in Henan, China, 2017–18: a test-negative case-control study. *The Lancet Child & Adolescent Health* **3,** 697–704 (2019).

64. Wang, X. *et al.* Enterovirus A71 vaccine effectiveness in preventing enterovirus A71 infection among medically-attended hand, foot, and mouth disease cases, Beijing, China. *Human vaccines & immunotherapeutics* **15,** 1183–1190 (2019).

65. Fang, C.-Y. & Liu, C.-C. Recent development of enterovirus A vaccine candidates for the prevention of hand, foot, and mouth disease. *Expert review of vaccines* **17,** 819–831 (2018).

66. Mao, Q., Wang, Y., Bian, L., Xu, M. & Liang, Z. EV-A71 vaccine licensure: a first step for multivalent enterovirus vaccine to control HFMD and other severe diseases. *Emerging Microbes & Infections* **5,** e75 (2016).

67. Chou, A.-H. *et al.* Formalin-inactivated EV71 vaccine candidate induced cross-neutralizing antibody against subgenotypes B1, B4, B5 and C4A in adult volunteers. *PLoS One* **8,** e79783 (2013).

68. Chang, Z., Liu, F., Bin, L., Wang, Z. & Zeng, L. Analysis on surveillance data of hand, foot and mouth disease in China, January-May 2017. *Disease Surveillance* **32,** 447–452 (2017).

69. Mathur, P. Hand hygiene: back to the basics of infection control. *The Indian journal of medical research* **134,** 611 (2011).

70. Ruan, F. *et al.* Risk factors for hand, foot, and mouth disease and herpangina and the preventive effect of hand-washing. *Pediatrics* **127,** e898–e904 (2011).

71. Chen, Y., Badaruddin, H., Lee, V. J., Cutter, J. & Cook, A. R. The effect of school closure on hand, foot, and mouth disease transmission in Singapore: a modeling approach. *The American journal of tropical medicine and hygiene* **99,** 1625–1632 (2018).

72. Hopkins, S. E. Acute flaccid myelitis: etiologic challenges, diagnostic and management considerations. *Current Treatment Options in Neurology* **19,** 48 (2017).

73. Van Haren, K. *et al.* Acute flaccid myelitis of unknown etiology in California, 2012-2015. *Jama* **314,** 2663–2671 (2015).

74. Romero, J. R. Pleconaril: a novel antipicornaviral drug. *Expert opinion on investigational drugs* **10,** 369–379 (2001).

75. Abzug, M. J. *et al.* A randomized, double-blind, placebo-controlled trial of pleconaril for the treatment of neonates with enterovirus sepsis. *Journal of the Pediatric Infectious Diseases Society* **5,** 53–62 (2016).

76. KEARNS, G. L. *et al.* Single dose pharmacokinetics of pleconaril in neonates. *The Pediatric infectious disease journal* **19,** 833–839 (2000).

77. Rotbart, H. A., Webster, A. D. & Group, P. T. R. Treatment of potentially life-threatening enterovirus infections with pleconaril. *Clinical infectious diseases* **32,** 228–235 (2001).

78. Hayden, F. G. *et al.* Efficacy and safety of oral pleconaril for treatment of colds due to picornaviruses in adults: results of 2 double-blind, randomized, placebo-controlled trials. *Clinical infectious diseases* **36,** 1523–1532 (2003).

79. McMinn, P. *et al.* Phylogenetic analysis of enterovirus 71 strains isolated during linked epidemics in Malaysia, Singapore, and Western Australia. *Journal of virology* **75,** 7732–7738 (2001).

80. Chen, S.-C., Chang, H.-L., Yan, T.-R., Cheng, Y.-T. & Chen, K.-T. An eight-year study of epidemiologic features of enterovirus 71 infection in Taiwan. *The American journal of tropical medicine and hygiene* **77,** 188–191 (2007).

81. Ang, L. W. *et al.* Epidemiology and control of hand, foot and mouth disease in Singapore. *Ann Acad Med Singapore* **38,** 106–12 (2009).

82. Wang, Y. *et al.* Hand, foot and mouth disease in China: patterns of spread and transmissibility during 2008-2009. *Epidemiology* **22,** 781–792 (2011).

83. Li, L. *et al.* Genetic characteristics of human enterovirus 71 and coxsackievirus A16 circulating from 1999 to 2004 in Shenzhen, People's Republic of China. *Journal of clinical microbiology* **43,** 3835–3839 (2005).

84. Yang, F. *et al.* Enterovirus 71 outbreak in the People's Republic of China in 2008. *Journal of clinical microbiology* **47,** 2351–2352 (2009).

85. Liu, Q. *et al.* Landscape of emerging and re-emerging infectious diseases in China: impact of ecology, climate, and behavior. *Frontiers of medicine* **12,** 3–22 (2018).

86. Ji, T. *et al.* Surveillance, epidemiology, and pathogen spectrum of hand, foot, and mouth disease in mainland of China from 2008 to 2017. *Biosafety and Health* (2019).

87. *Western Pacific region hand, foot and mouth disease (HFMD) surveillance summary* http://www.wpro.who.int/emerging_diseases/HFMD/en/.

88. Messacar, K. *et al.* Resource burden during the 2014 enterovirus D68 respiratory disease outbreak at Children's Hospital Colorado: an unexpected strain. *JAMA Pediatrics* **170,** 294–297 (2016).

89. *2017 Annual review of diseases prioritized under the Research and Development Blueprint* (World Health Organisation, Geneva,Switzerland, 2017). http://www.who.int/blueprint/what/research-development/2017-Prioritization-Long-Report.pdf.

90. *2018 Annual review of diseases prioritized under the Research and Development Blueprint* (World Health Organisation, Geneva,Switzerland, 2018). http://www.who.int/emergencies/diseases/2018prioritization-report.pdf.

91. Oberste, M. S., Maher, K., Kilpatrick, D. R. & Pallansch, M. A. Molecular evolution of the human enteroviruses: correlation of serotype with VP1 sequence and application to picornavirus classification. *Journal of Virology* **73,** 1941–1948 (1999).

92. Oberste, M. S. *et al.* Typing of human enteroviruses by partial sequencing of VP1. *Journal of clinical microbiology* **37,** 1288–1293 (1999).

93. Ishiko, H. *et al.* Molecular diagnosis of human enteroviruses by phylogeny-based classification by use of the VP4 sequence. *The Journal of infectious diseases* **185,** 744–754 (2002).

94. Oberste, M. S., Maher, K. & Pallansch, M. A. Molecular phylogeny and proposed classification of the simian picornaviruses. *Journal of virology* **76,** 1244–1251 (2002).

95. Lee, T., Megson, B. & Kurtz, J. Enterovirus typing by immune electronmicroscopy. *Journal of medical microbiology* **44,** 151–153 (1996).

96. Van Loon, A., Cleator, G. & Ras, A. External quality assessment of enterovirus detection and typing. European Union Concerted Action on Virus Meningitis and Encephalitis. *Bulletin of the World Health Organization* **77,** 217 (1999).

97. Fauquet, C. M., Mayo, M. A., Maniloff, J., Desselberger, U. & Ball, L. A. *Virus taxonomy: eighth report of the International Committee on Taxonomy of Viruses* (Academic Press, 2005).

98. *Batch geocoding* https://www.findlatitudeandlongitude.com/batch-geocode/. Accessed 26 Sep 2019.

99. Elliott, P. & Wartenberg, D. Spatial epidemiology: current approaches and future challenges. *Environmental health perspectives* **112,** 998–1006 (2004).

100. Oberste, M. S. *et al.* Molecular identification of 13 new enterovirus types, EV79–88, EV97, and EV100–101, members of the species Human Enterovirus B. *Virus research* **128,** 34–42 (2007).

101. Oberste, M. S. & Pallansch, M. A. Enterovirus molecular detection and typing. *Reviews in Medical Microbiology* **16,** 163–171 (2005).

102. Oberste, M. S., Jiang, X., Maher, K., Nix, W. A. & Jiang, B. The complete genome sequences for three simian enteroviruses isolated from captive primates. *Archives of virology* **153,** 2117–2122 (2008).

103. Oberste, M. S. *et al.* Comparison of classic and molecular approaches for the identification of untypeable enteroviruses. *Journal of Clinical Microbiology* **38,** 1170–1174 (2000).

104. Nikolaidis, M. *et al.* Large-scale genomic analysis reveals recurrent patterns of intertypic recombination in human enteroviruses. *Virology* **526,** 72–80 (2019).

105. McMinn, P. C. An overview of the evolution of enterovirus 71 and its clinical and public health significance. *FEMS microbiology reviews* **26,** 91–107 (2002).

106. Fieldhouse, J. K., Wang, X., Mallinson, K. A., Tsao, R. W. & Gray, G. C. A systematic review of evidence that enteroviruses may be zoonotic. *Emerging microbes & infections* **7,** 1–09 (2018).

107. Lloyd-Smith, J. O. *et al.* Epidemic dynamics at the human-animal interface. *science* **326,** 1362–1367 (2009).

108. Palmer, S., Brown, D. & Morgan, D. Early qualitative risk assessment of the emerging zoonotic potential of animal diseases. *BMJ* **331,** 1256–1260 (2005).

109. Lukashev, A. N. & Vakulenko, Y. A. Molecular evolution of types in non-polio enteroviruses. *Journal of General Virology* **98,** 2968–2981 (2017).

110. Eshaghi, A. *et al.* Global distribution and evolutionary history of enterovirus D68, with emphasis on the 2014 outbreak in Ontario, Canada. *Frontiers in microbiology* **8,** 257 (2017).

111. Oberste, M. S. *et al.* Enteroviruses 76, 89, 90 and 91 represent a novel group within the species Human enterovirus A. *Journal of general virology* **86,** 445–451 (2005).

112. Smura, T. *et al.* Enterovirus surveillance reveals proposed new serotypes and provides new insight into enterovirus 5-untranslated region evolution. *Journal of general virology* **88,** 2520–2526 (2007).

113. Moore, M. Enteroviral disease in the United States, 1970-1979. *The Journal of infectious diseases* **146,** 103–108 (1982).

114. Organisation, W. H. *WHO-recommended surveillance standard of poliomyelitis* https://www.who.int/immunization/monitoring_surveillance/burden/vpd/surveillance_type/active/poliomyelitis_standards/en/.

115. Sadeuh-Mba, S. A. *et al.* Genetic and phenotypic characterization of recently discovered enterovirus D type 111. *PLoS neglected tropical diseases* **13,** e0007797 (2019).

116. Muir, P. *et al.* Molecular typing of enteroviruses: current status and future requirements. *Clinical microbiology reviews* **11,** 202–227 (1998).

117. Brown, B. A. *et al.* Resolving ambiguities in genetic typing of human enterovirus species C clinical isolates and identification of enterovirus 96, 99 and 102. *Journal of General Virology* **90,** 1713–1723 (2009).

118. Jorba, J., Campagnoli, R., De, L. & Kew, O. Calibration of multiple poliovirus molecular clocks covering an extended evolutionary range. *Journal of virology* **82,** 4429–4440 (2008).

119. Oberste, M. S. in *Group B Coxsackieviruses* 33–47 (Springer, 2008).

120. David, P. M. & Le Dévédec, N. Preparedness for the next epidemic: health and political issues of an emerging paradigm. *Critical Public Health,* 1–7 (2018).

121. Lakoff, A. Preparing for the next emergency. *Public Culture* **19,** 247–271 (2007).

122. Weber, S. G., Bottei, E., Cook, R. & O'Connor, M. SARS, emerging infections, and bioterrorism preparedness. *The Lancet Infectious Diseases* **4,** 483–484 (2004).

123. Lancet, T. Public-health preparedness requires more than surveillance. *The Lancet* **364,** 1639–1640 (2004).

124. ECDC. *Guide to revision of national pandemic influenza preparedness plans: Lessons learned from the 2009 A(H1N1) pandemic* (European Centre for Disease Prevention and Control, Stockholm, Sweden, 2016).

125. Organization, W. H. *et al. WHO checklist for influenza pandemic preparedness planning* tech. rep. (World Health Organization, 2005).

126. Monday, B. *et al.* Paradigm shift: contribution of field epidemiology training in advancing the "One Health" approach to strengthen disease surveillance and outbreak investigations in Africa. *The Pan African medical journal* **10 Supp 1** (2011).

127. Coker, R. & Mounier-Jack, S. Pandemic influenza preparedness in the Asia–Pacific region. *The Lancet* **368,** 886–889 (2006).

128. Jennings, L. C., Monto, A. S., Chan, P. K., Szucs, T. D. & Nicholson, K. G. Stockpiling prepandemic influenza vaccines: a new cornerstone of pandemic preparedness plans. *The Lancet Infectious Diseases* **8,** 650–658 (2008).

129. Perkins, M. D. *et al.* Diagnostic preparedness for infectious disease outbreaks. *The Lancet* **390,** 2211–2214 (2017).

130. Salama, P. WHO R&D Blueprint: a global coordination mechanism for R&D preparedness. *The Lancet* **389,** 2469–2470 (2017).

131. Quaglio, G. *et al.* Ebola: lessons learned and future challenges for Europe. *The Lancet Infectious Diseases* **16,** 259–263 (2016).

132. Sands, P. & Chawla, M. Financing preparedness at a national level. *The Lancet* **389,** 2086–2087 (2017).

133. Olliaro, P. L. Initiation and publication time-lags of treatment trials for Ebola virus disease. *The Lancet Infectious Diseases* **18,** 28–29 (2018).

134. O'Brien, E., Taft, R., Geary, K., Ciotti, M. & Suk, J. Best practices in ranking communicable disease threats: a literature review, 2015. *Eurosurveillance* **21** (2016).

135. Pigott, D. M. *et al.* Prioritising infectious disease mapping. *PLoS Neglected Tropical Diseases* **9,** e0003756 (2015).

136. Cox, R. *et al.* Comparison of the h-index scores among pathogens identified as emerging hazards in North America. *Transboundary and Emerging Diseases* **63,** 79–91 (2016).

137. Hay, S. I. *et al.* Global mapping of infectious disease. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **368.** http://rstb.royalsocietypublishing.org/content/368/1614/20120250 (2013).

138. *The Dubrovnik pledge on surveillance and prioritization of infectious diseases* (World Health Organisation, Bucharest, Romania, 2002). http://apps.who.int/iris/handle/10665/107469.

139. Humblet, M. F. *et al.* Multidisciplinary and evidence-based method for prioritizing diseases of food-producing animals and zoonoses. *Emerging Infectious Diseases* **18,** e1 (2012).

140. Trock, S. C., Burke, S. A. & Cox, N. J. Development of an influenza virologic risk assessment tool. *Avian Diseases* **56,** 1058–1061 (2012).

141. Burke, S. A. & Trock, S. C. Use of Influenza Risk Assessment Tool for Prepandemic Preparedness. *Emerging Infectious Diseases* **24,** 471–477 (2018).

142. *Tool for influenza pandemic risk assessment (TIPRA)* (World Health Organization, Geneva, Switzerland, 2016).

143. Dodgson, J. S., Spackman, M., Pearman, A. & Phillips, L. D. Multi-criteria analysis: a manual (2009).

144. Greco, S., Figueira, J. & Ehrgott, M. *Multiple criteria decision analysis* (Springer, 2016).

145. Angelis, A. & Kanavos, P. Multiple criteria decision analysis (MCDA) for evaluating new medicines in health technology assessment and beyond: the Advance Value Framework. *Social Science & Medicine* **188,** 137–156 (2017).

146. *ECDC tool for the prioritisation of infectious disease threats* (European Centre for Disease Control and Prevention, Stockholm, Sweden, 2017). https://ecdc.europa.eu/sites/portal/files/documents/Tool-for-disease-priority-ranking_handbook_0_0.pdf.

147. Hsu, C.-C. & Sandford, B. A. The Delphi technique: making sense of consensus. *Practical Assessment, Research & Evaluation* **12,** 1–8 (2007).

148. Etikan, I., Musa, S. A. & Alkassim, R. S. Comparison of convenience sampling and purposive sampling. *American Journal of Theoretical and Applied Statistics* **5,** 1–4 (2016).

149. *Genus: Enterovirus* https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/picornavirales/w/picornaviridae/681/genus-enterovirus.

150. McIntyre, K. M., Hawkes, I., Waret-Szkuta, A., Morand, S. & Baylis, M. The H-index as a quantitative indicator of the relative impact of human diseases. *PLoS One* **6,** e19558 (2011).

151. Roszkowska, E. Rank ordering criteria weighting methods–a comparative overview. *Optimum. Studia Ekonomiczne* **5,** 14–33 (2013).

152. Barron, F. H. & Barrett, B. E. Decision quality using ranked attribute weights. *Management science* **42,** 1515–1523 (1996).

153. Edwards, W. & Barron, F. H. SMARTS and SMARTER: Improved simple methods for multiattribute utility measurement. *Organizational behavior and human decision processes* **60,** 306–325 (1994).

154. Dodgson, J. S., Spackman, M., Pearman, A. & Phillips, L. D. Multi-criteria analysis: a manual (2009).

155. Nutt, D. J., King, L. A., Phillips, L. D., *et al.* Drug harms in the UK: a multicriteria decision analysis. *The Lancet* **376,** 1558–1565 (2010).

156. Kao, C. Weight determination for consistently ranking alternatives in multiple criteria decision analysis. *Applied Mathematical Modelling* **34,** 1779–1787 (2010).

157. Taber, K. S. The use of Cronbach's alpha when developing and reporting research instruments in science education. *Research in Science Education* **48,** 1273–1296 (2018).

158. Braun, V. & Clarke, V. *Thematic analysis* 843–860 (Springer, 2019).

159. Elliott, V. Thinking about the coding process in qualitative data analysis. *Qualitative Report* **23** (2018).

160. Braun, V. & Clarke, V. Using thematic analysis in psychology. *Qualitative research in psychology* **3,** 77–101 (2006).

161. Srivastava, A. & Thomson, S. B. Framework analysis: a qualitative methodology for applied policy research (2009).

162. Gale, N. K., Heath, G., Cameron, E., Rashid, S. & Redwood, S. Using the framework method for the analysis of qualitative data in multi-disciplinary health research. *BMC medical research methodology* **13,** 117 (2013).

163. Cox, R., Revie, C. W. & Sanchez, J. The use of expert opinion to assess the risk of emergence or re-emergence of infectious diseases in Canada associated with climate change. *PLoS One* **7,** e41590 (2012).

164. Cox, R., Sanchez, J. & Revie, C. W. Multi-criteria decision analysis tools for prioritising emerging or re-emerging infectious diseases associated with climate change in Canada. *PLoS One* **8,** e68338 (2013).

165. Balabanova, Y. *et al.* Communicable diseases prioritized for surveillance and epidemiological research: results of a standardized prioritization procedure in Germany, 2011. *PloS One* **6,** e25691 (2011).

166. Cardoen, S. *et al.* Evidence-based semiquantitative methodology for prioritization of foodborne zoonoses. *Foodborne Pathogens and Disease* **6,** 1083–1096 (2009).

167. Havelaar, A. H. *et al.* Prioritizing emerging zoonoses in the Netherlands. *PloS One* **5,** e13965 (2010).

168. Morgan, D., Kirkbride, H., Hewitt, K., Said, B. & Walsh, A. Assessing the risk from emerging infections. *Epidemiology & Infection* **137,** 1521–1530 (2009).

169. Trock, S. C., Burke, S. A. & Cox, N. J. Development of framework for assessing influenza virus pandemic risk. *Emerging Infectious Diseases* **21,** 1372 (2015).

170. Garner, M. J. *et al.* An assessment of antimicrobial resistant disease threats in Canada. *PLoS One* **10,** e0125155 (2015).

171. Economopoulou, A., Kinross, P., Domanovic, D. & Coulombier, D. Infectious diseases prioritisation for event-based surveillance at the European Union level for the 2012 Olympic and Paralympic Games. *Eurosurveillance* **19,** 20770 (2014).

172. Gilsdorf, A. & Krause, G. Prioritisation of infectious diseases in public health: feedback on the prioritisation methodology, 15 July 2008 to 15 January 2009 (2011).

173. Krause, G. Prioritisation of infectious diseases in public health-call for comments (2008).

174. McKenzie, J., Simpson, H. & Langstaff, I. Development of methodology to prioritise wildlife pathogens for surveillance. *Preventive Veterinary Medicine* **81,** 194–210 (2007).

175. Van Lier, A. *et al.* Disease burden of 32 infectious diseases in the Netherlands, 2007-2011. *PloS One* **11,** e0153106 (2016).

176. McIntyre, K. M. *et al.* A quantitative prioritisation of human and domestic animal pathogens in Europe. *PLoS One* **9,** e103529 (2014).

177. England, P. H. *Risk assessment of enterovirus D68s,* (Public Health England, London, United Kingdom, 2018).

178. NikNadia, N. *et al.* Cyclical patterns of hand, foot and mouth disease caused by enterovirus A71 in Malaysia. *PLoS Neglected Tropical Diseases* **10,** e0004562 (2016).

179. *Surveillance Guide for Vaccine-Preventable Diseases in the WHO South-East Asia Region* (World Health Organization et al., 2017).

180. Muehlenbachs, A., Bhatnagar, J. & Zaki, S. R. Tissue tropism, pathology and pathogenesis of enterovirus infection. *The Journal of Pathology* **235,** 217–228 (2015).

181. Yang, B. *et al.* Seroprevalence of enterovirus 71 antibody among children in China: a systematic review and meta-analysis. *The Pediatric Infectious Disease Journal* **34,** 1399 (2015).

182. Wang, J.-x. *et al.* Seroprevalence of Enterovirus A71 and Coxsackievirus A16 in Healthy People in Shandong Province, China. *PloS One* **11,** e0162373 (2016).

183. Wu, T.-C. *et al.* Immunity to avirulent enterovirus 71 and coxsackie A16 virus protects against enterovirus 71 infection in mice. *Journal of Virology* **81,** 10310–10315 (2007).

184. Huang, W.-C., Huang, L.-M., Lu, C.-Y., Cheng, A.-L. & Chang, L.-Y. Atypical hand-foot-mouth disease in children: a hospital-based prospective cohort study. *Virology journal* **10,** 209 (2013).

185. Chen, Y. *et al.* Antigenic analysis of divergent genotypes human Enterovirus 71 viruses by a panel of neutralizing monoclonal antibodies: current genotyping of EV71 does not reflect their antigenicity. *Vaccine* **31,** 425–430 (2013).

186. Yee, P. T. I. & Poh, C. L. Impact of genetic changes, pathogenicity and antigenicity on Enterovirus-A71 vaccine development. *Virology* **506,** 121–129 (2017).

187. Tan, C. W., Lai, J. K. F., Sam, I.-C. & Chan, Y. F. Recent developments in antiviral agents against enterovirus 71 infection. *Journal of Biomedical Science* **21,** 14 (2014).

188. Russell, C. A. *et al.* Improving pandemic influenza risk assessment. *Elife* **3** (2014).

189. Mandary, M. & Poh, C. Changes in the EV-A71 Genome through Recombination and Spontaneous Mutations: Impact on Virulence. *Viruses* **10,** 320 (2018).

190. Lin, J.-Y. & Shih, S.-R. Cell and tissue tropism of enterovirus 71 and other enteroviruses infections. *Journal of Biomedical Science* **21,** 18 (2014).

191. *Best practices in ranking emerging infectious disease threats.* (European Centre for Disease Control and Prevention, Stockholm, Sweden, 2015). https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/emerging-infectious-disease-threats-best-practices-ranking.pdf.

192. Imamura, T. & Oshitani, H. Global reemergence of enterovirus D68 as an important pathogen for acute respiratory infections. *Reviews in Medical Virology* **25,** 102–114 (2015).

193. Kapusinszky, B., Szomor, K. N., Farkas, Á., Takács, M. & Berencsi, G. Detection of non-polio enteroviruses in Hungary 2000–2008 and molecular epidemiology of enterovirus 71, coxsackievirus A16, and echovirus 30. *Virus genes* **40,** 163–173 (2010).

194. Gaaloul, I. *et al.* Coxsackievirus B detection in cases of myocarditis, myopericarditis, pericarditis and dilated cardiomyopathy in hospitalized patients. *Molecular Medicine Reports* **10,** 2811–2818 (2014).

195. Anh, N. T. *et al.* Emerging Coxsackievirus A6 Causing Hand, Foot and Mouth Disease, Vietnam. *Emerging Infectious Diseases* **24,** 654 (2018).

196. Second, J. *et al.* Clinicopathologic analysis of atypical hand, foot, and mouth disease in adult patients. *Journal of the American Academy of Dermatology* **76,** 722–729 (2017).

197. Drago, F., Ciccarese, G., Broccolo, F., Rebora, A. & Parodi, A. Atypical hand, foot, and mouth disease in adults. *Journal of the American Academy of Dermatology* **77,** e51–e56 (2017).

198. Baruch, Y. & Holtom, B. C. Survey response rate levels and trends in organizational research. *Human Relations* **61,** 1139–1160 (2008).

199. DeMaio, T. J. Refusals: Who, where and why. *Public Opinion Quarterly* **44,** 223–233 (1980).

200. Cheung, K. L., Peter, M., Smit, C., de Vries, H. & Pieterse, M. E. The impact of non-response bias due to sampling in public health studies: a comparison of voluntary versus mandatory recruitment in a Dutch national survey on adolescent health. *BMC public health* **17,** 1–10 (2017).

201. Saunders, B. *et al.* Saturation in qualitative research: exploring its conceptualization and operationalization. *Quality & Quantity* **52,** 1893–1907 (2018).

202. Corvest, V. *et al.* Fatal case of enterovirus A71 hand, foot, and mouth disease infection. *Archives de pediatrie* **24,** 1253–1258 (2017).

203. Rahimi, R., Omar, E., Soh, T. S. T., Nawi, S. F. A. M. & Noor, S. M. A sudden paediatric death due to hand, foot and mouth disease: the importance of vigilance. *The Malaysian journal of pathology* **39,** 167 (2017).

204. Xing, J., Wang, K., Wei, H. & Wei, D. Pathologic and molecular studies of enterovirus 71 infection in a fatal case from a recent epidemic in China: A case report. *Medicine* **97** (2018).

205. Qui, P. T. *et al.* Intravenous magnesium sulfate for the management of severe hand, foot, and mouth disease with autonomic nervous system dysregulation in Vietnamese children: study protocol for a randomized controlled trial. *Trials* **17,** 98 (2016).

206. Li, J. *et al.* Epidemiological surveillance of hand, foot and mouth disease in Shanghai in 2014-2016, prior to the introduction of the enterovirus 71 vaccine. *Emerging Microbes & Infections* **7,** 37 (2018).

207. Yu, S. B. *et al.* Hospitalization burden of hand, foot and mouth disease in Anhua county of Hunan province, 2013-2016. *Zhonghua Liu Xing Bing Xue Za Zhi* **40,** 79–83 (2019).

208. Yu, S. *et al.* Population based hospitalization burden of laboratory-confirmed hand, foot and mouth disease caused by multiple enterovirus serotypes in Southern China. *PLoS ONE* **13,** e0203792 (2018).

209. Qiu, J. *et al.* the clinical and epidemiological Study of children with Hand, foot, and Mouth Disease in Hunan, china from 2013 to 2017. *Scientific reports* **9** (2019).

210. Cai, K. *et al.* Clinical characteristics and managements of severe hand, foot and mouth disease caused by enterovirus A71 and coxsackievirus A16 in Shanghai, China. *BMC Infectious Diseases* **19,** 285 (2019).

211. Owatanapanich, S., Wutthanarungsan, R., Jaksupa, W. & Thisyakorn, U. Risk Factors for Severe Enteroviral Infections in Children. *J Med Assoc Thai* **99,** 322–330 (2016).

212. Kim, B. *et al.* Factors associated with severe neurologic complications in patients with either hand-foot-mouth disease or herpangina: A nationwide observational study in South Korea, 2009-2014. *PLoS ONE* **13,** e0201726 (2018).

213. Crabol, Y. *et al.* A prospective, comparative study of severe neurological and uncomplicated hand, foot and mouth forms of paediatric enterovirus 71 infections. *Int. J. Infect. Dis.* **59,** 69–76 (2017).

214. Hoang, M. T. V. *et al.* Clinical and aetiological study of hand, foot and mouth disease in southern Vietnam, 2013-2015: Inpatients and outpatients. *Int. J. Infect. Dis.* **80,** 1–9 (2019).

215. Liu, G. *et al.* Developing a Machine Learning System for Identification of Severe Hand, Foot, and Mouth Disease from Electronic Medical Record Data. *Sci Rep* **7,** 16341 (2017).

216. Jones, E. *et al.* Outcomes following severe hand foot and mouth disease: A systematic review and meta-analysis. *Eur. J. Paediatr. Neurol.* **22,** 763–773 (2018).

217. Zheng, G. *et al.* Risk factors for death in children with critical and severe hand-foot-and-mouth disease in Chongqing, China: An observational study. *Medicine (Baltimore)* **96,** e8934 (2017).

218. Hu, Y., Jiang, L. & Peng, H. L. Clinical Analysis of 134 Children with Nervous System Damage Caused by Enterovirus 71 Infection. *Pediatr. Infect. Dis. J.* **34,** 718–723 (2015).

219. Jiao, W. *et al.* The Effectiveness of Different Doses of Intravenous Immunoglobulin on Severe Hand, Foot and Mouth Disease: A Meta-Analysis. *Med Princ Pract* **28,** 256–263 (2019).

220. Chea, S. *et al.* Workshop on use of intravenous immunoglobulin in hand, foot and mouth disease in Southeast Asia. *Emerging Infect. Dis.* **21** (2015).

221. Matt, V. & Matthew, H. The retrospective chart review: important methodological considerations. *Journal of educational evaluation for health professions* **10** (2013).

222. Gearing, R. E., Mian, I. A., Barber, J. & Ickowicz, A. A methodology for conducting retrospective chart review research in child and adolescent psychiatry. *Journal of the Canadian Academy of Child and Adolescent Psychiatry* **15,** 126 (2006).

223. Vincent, C., Neale, G. & Woloshynowych, M. Adverse events in British hospitals: preliminary retrospective record review. *BMJ* **322,** 517–519 (2001).

224. Fain, J. M. *et al.* A multicenter, retrospective chart review study comparing index therapy change rates in open-angle glaucoma or ocular hypertension patients newly treated with latanoprost or travoprost-Z monotherapy. *BMC ophthalmology* **11,** 13 (2011).

225. Worster, A. & Haines, T. Advanced statistics: understanding medical record review (MRR) studies. *Academic Emergency Medicine* **11,** 187–192 (2004).

226. Karyanti, M. R. *et al.* Clinical Course and Management of Dengue in Children Admitted to Hospital; A 5 Years Prospective Cohort Study in Jakarta, Indonesia. *The Pediatric infectious disease journal* (2019).

227. For Disease Control, C. C. & Prevention. *National hand-foot-mouth disease sruveillance data in China* 2019. http://www.phsciencedata.cn/Share/.

228. Garg, R. Methodology for research I. *Indian Journal of Anaesthesia* **60,** 640 (2016).

229. Patino, C. M. & Ferreira, J. C. Inclusion and exclusion criteria in research studies: definitions and why they matter. *Jornal Brasileiro de Pneumologia* **44,** 84 (2018).

230. Graham, C. Anonymisation: managing data protection risk code of practice. *Information Commissioner's Office* (2012).

231. Of Health, U. D., Services, H., *et al.* Guidance regarding methods for de-identification of protected health information in accordance with the Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule. *US Department of Health and Human Services, Washington, DC) Available at: https://www. hhs. gov/hipaa/for-professionals/privacy/special-topics/de-identification/index. html. Accessed September* **26,** 2018 (2012).

232. Li, L.-Q. *et al.* Development of EV71, CA16 and other enterovirus vrial real-time qualitative PCR diagnostic kit. *Chinese journal of experimental and clinical virology* **27,** 224–227 (2013).

233. *Standards for Assessing, Measuring and Monitoring Vital Signs in Infants, Children and Young People* (Royal College of Nursing, 2017). https://www.rcn.org.uk/professional-development/publications/pub-005942.

234. *Clinical Biochemistry Reference Ranges Handbook* (East Sussex Healthcare NHS Trust, 2018).

235. Data protection act. *London Station Off* (1998).

236. Van Veen, E.-B. Observational health research in Europe: understanding the General Data Protection Regulation and underlying debate. *European Journal of Cancer* **104,** 70–80 (2018).

237. *Information security technology and personal information security specifications* (The Standardization Administration of the People's Republic of China, 2019). https://www.secrss.com/articles/14053.

238. Bellary, S., Krishnankutty, B. & Latha, M. Basics of case report form designing in clinical research. *Perspectives in Clinical Research* **5,** 159 (2014).

239. Whitney, C. W., Lind, B. K. & Wahl, P. W. Quality assurance and quality control in longitudinal studies. *Epidemiologic reviews* **20,** 71–80 (1998).

240. Pandav, R. *et al.* Data management and quality assurance for an International project: the Indo–US Cross-National Dementia Epidemiology Study. *International journal of geriatric psychiatry* **17,** 510–518 (2002).

241. Messacar, K. *et al.* Clinical characteristics of enterovirus A71 neurological disease during an outbreak in children in Colorado, USA, in 2018: an observational cohort study. *The Lancet Infectious Diseases* **20,** 230–239 (2020).

242. Cui, P. *et al.* Clinical analysis of 555 outpatients with hand, foot and mouth diseases caused by different enteroviruses. *Chinese Journal of Pediatrics* **57,** 445 (2019).

243. *Public health science data-hand, foot and mouth disease.* 2020. http://www.phsciencedata.cn/Share/.

244. Huang, C.-C. *et al.* Neurologic complications in children with enterovirus 71 infection. *New England Journal of Medicine* **341,** 936–942 (1999).

245. Lin, T.-Y. *et al.* The 1998 enterovirus 71 outbreak in Taiwan: pathogenesis and management. *Clinical Infectious Diseases* **34,** S52–S57 (2002).

246. Jiang, M. & Wang, X. C. Clinical research of early, enough methylprednisone combined with immunoglobulin in treatment of severe hand-foot-mouth disease. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi* **27,** 363–365 (2013).

247. Abebe, F. E., Gebeyehu, A. W., Kidane, A. N. & Eyassu, G. A. Factors leading to cesarean section delivery at Felegehiwot referral hospital, Northwest Ethiopia: a retrospective record review. *Reproductive health* **13,** 6 (2015).

248. Aschengrau, A. & Seage, G. R. *Essentials of epidemiology in public health* (Jones & Bartlett Publishers, 2013).

249. Skelly, A. C., Dettori, J. R. & Brodt, E. D. Assessing bias: the importance of considering confounding. *Evidence-based spine-care journal* **3,** 9 (2012).

250. Zeng, M. *et al.* Epidemiology of hand, foot, and mouth disease in children in Shanghai 2007–2010. *Epidemiology & Infection* **140,** 1122–1130 (2012).

251. Ji, H. *et al.* Surveillance for severe hand, foot, and mouth disease from 2009 to 2015 in Jiangsu province: epidemiology, etiology, and disease burden. *BMC Infectious Diseases* **19,** 79 (2019).

252. Chang, L.-Y. *et al.* Clinical features and risk factors of pulmonary oedema after enterovirus-71-related hand, foot, and mouth disease. *The Lancet* **354,** 1682–1686 (1999).

253. Ho, M. *et al.* An epidemic of enterovirus 71 infection in Taiwan. *New England Journal of Medicine* **341,** 929–935 (1999).

254. Experts consensus on rescue and treatment of severe cases with enterovirus 71 (EV71) infection. *Chin J Pediatr* **49,** 675–8 (2011).

255. Li, X.-W. *et al.* Chinese guidelines for the diagnosis and treatment of hand, foot and mouth disease (2018 edition). *World Journal of Pediatrics* **14,** 437–447 (2018).

256. *Flow chart for diagnosis and treatment of HFMD in children* (Department of Health, People's Committee of Ho Chi Minh City, 2012).

257. Phuong, C. X. T. *et al.* Clinical diagnosis and assessment of severity of confirmed dengue infections in Vietnamese children: is the World Health Organization classification system helpful? *The American journal of tropical medicine and hygiene* **70,** 172–179 (2004).

258. Phuong, C. X. *et al.* Clinical diagnosis and assessment of severity of confirmed dengue infections in Vietnamese children: is the world health organization classification system helpful? *Am. J. Trop. Med. Hyg.* **70,** 172–179 (2004).

259. Bandyopadhyay, S., Lum, L. C. & Kroeger, A. Classifying dengue: a review of the difficulties in using the WHO case classification for dengue haemorrhagic fever. *Trop. Med. Int. Health* **11,** 1238–1255 (2006).

260. Macedo, G. A. *et al.* Sensitivity and specificity of the World Health Organization dengue classification schemes for severe dengue assessment in children in Rio de Janeiro. *PloS one* **9,** e96314 (2014).

261. Horstick, O. *et al.* Comparing the usefulness of the 1997 and 2009 WHO dengue case classification: a systematic literature review. *The American journal of tropical medicine and hygiene* **91,** 621–634 (2014).

262. Macedo, G. A. *et al.* Sensitivity and specificity of the World Health Organization dengue classification schemes for severe dengue assessment in children in Rio de Janeiro. *PLoS ONE* **9,** e96314 (2014).

263. Horstick, O. *et al.* Comparing the usefulness of the 1997 and 2009 WHO dengue case classification: a systematic literature review. *Am. J. Trop. Med. Hyg.* **91,** 621–634 (2014).

264. Narvaez, F. *et al.* Evaluation of the traditional and revised WHO classifications of Dengue disease severity. *PLoS Neglected Tropical Diseases* **5,** e1397 (2011).

265. Landis, J. R. & Koch, G. G. The measurement of observer agreement for categorical data. *Biometrics,* 159–174 (1977).

266. Chang, L.-Y. *et al.* Outcome of enterovirus 71 infections with or without stage-based management: 1998 to 2002. *The Pediatric infectious disease journal* **23,** 327–332 (2004).

267. *Hand foot mouth disease (HFMD) guidelines* (The Ministry of Health Maylaysia, 2007). www.moh.gov.my%20%E2%80%BA%20index.php%20%E2%80%BA%20file_manager%20%E2%80%BA%20dl_item. Accessed 9 Sept 2018.

268. Sabanathan, S. *Neurodevelopmental Outcomes Following Severe Hand Foot and Mouth Disease in Vietnam* PhD thesis (Open University (United Kingdom), 2016).

269. Solomon, T. *et al.* A cohort study to assess the new WHO Japanese encephalitis surveillance standards. *Bulletin of the World Health Organization* **86,** 178–186 (2008).

270. *Guideline on the diagnosis and clinical management for hand-foot-mouth disease (2010)* (China Ministry of Health, China, Beijing, 2010). http://www.nhfpc.gov.cn/mohyzs/s3586/201004/46884.shtml.

271. *Consensus clinical management guideline for EV-A71 caused severe HFMD (2011)* (China Ministry of Health, China, Beijing, 2011). www.moh.gov.cn/cmsresources/mohyzs/cmsrsdocument/doc11994.doc.

272. De Bruijn, M. A. *et al.* Long-term neuropsychological outcome following pediatric anti-NMDAR encephalitis. *Neurology* **90,** e1997–e2005 (2018).

273. Zhang, L.-n. *et al.* Epidemiological features and risk factors of sepsis-associated encephalopathy in intensive care unit patients: 2008-2011. *Chinese Medical Journal* **125,** 828–831 (2012).

274. Koh, W. M. *et al.* Severity and burden of hand, foot and mouth disease in Asia: a modelling study. *BMJ global health* **3,** e000442 (2018).

275. Price, J., Kassam-Adams, N., Alderfer, M. A., Christofferson, J. & Kazak, A. E. Systematic review: A reevaluation and update of the integrative (trajectory) model of pediatric medical traumatic stress. *Journal of Pediatric Psychology* **41,** 86–97 (2015).

276. James, S. L. *et al.* Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet* **392,** 1789–1858 (2018).

277. Lin, J.-Y., Kung, Y.-A. & Shih, S.-R. Antivirals and vaccines for Enterovirus A71. *Journal of biomedical science* **26,** 65 (2019).

278. Chung, K. C., Song, J. W., Group, W. S., *et al.* A guide on organizing a multicenter clinical trial: the WRIST study group. *Plastic and reconstructive surgery* **126,** 515 (2010).

279. Schlapbach, L. J. *et al.* Enteroviral myocarditis in neonates. *Journal of paediatrics and child health* **49,** E451–E454 (2013).

280. Turner, M. A. Clinical trials of medicines in neonates: the influence of ethical and practical issues on design and conduct. *British journal of clinical pharmacology* **79,** 370–378 (2015).

281. Caldwell, P. H., Murphy, S. B., Butow, P. N. & Craig, J. C. Clinical trials in children. *The Lancet* **364,** 803–811 (2004).

282. Bernstein, S. L. & Feldman, J. Incentives to participate in clinical trials: Practical and ethical considerations. *The American journal of emergency medicine* **33,** 1197–1200 (2015).