UNIVERSITI MALAYA

Epidemiology of Hand, Foot and Mouth Disease in South-East Asia & Key Immunological Considerations for Vaccine Development

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31 October - 1 November 2023, WHO/MPP mRNA regional workshop in Bangkok, Thailand

Hand, Foot and Mouth Disease (HFMD)

General

- Highly contagious
- Common in young children
- Group of enteroviruses- **coxsackievirus A viruses, enterovirus A71, echoviruses**
- Pathogenesis
  - faecal-oral, direct
  - replicate in oropharynx
  - viraemia and dissemination to target organs (CNS, skin)
  - excreted in pharynx and faeces for weeks

Symptoms

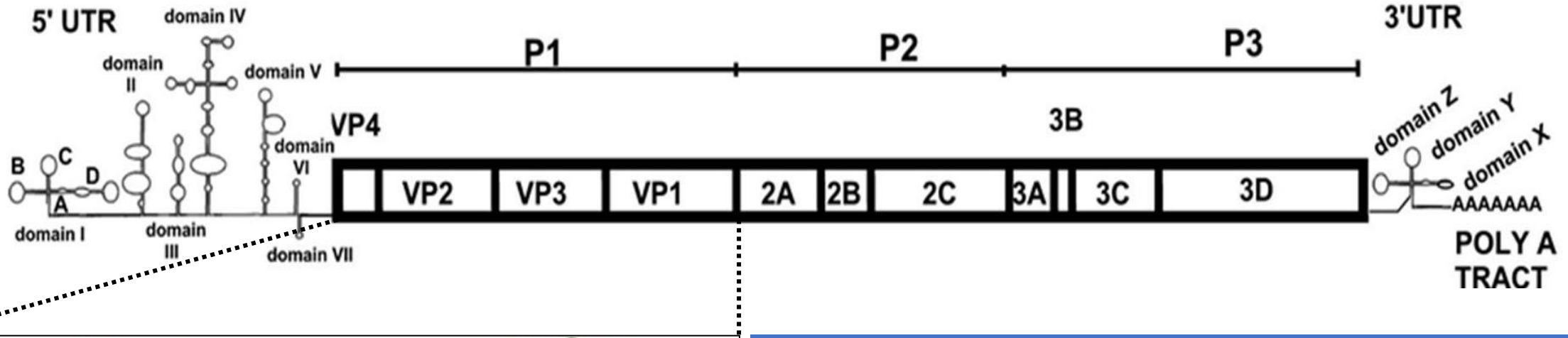
- Fever, sore throat, mouth ulcers
- Herpangina vs HFMD
- Blisters on palms of hands and soles of feet
- Symptoms usually appear 3 to 5 days after exposure
- Recurrent HFMD- 0.45%[4]

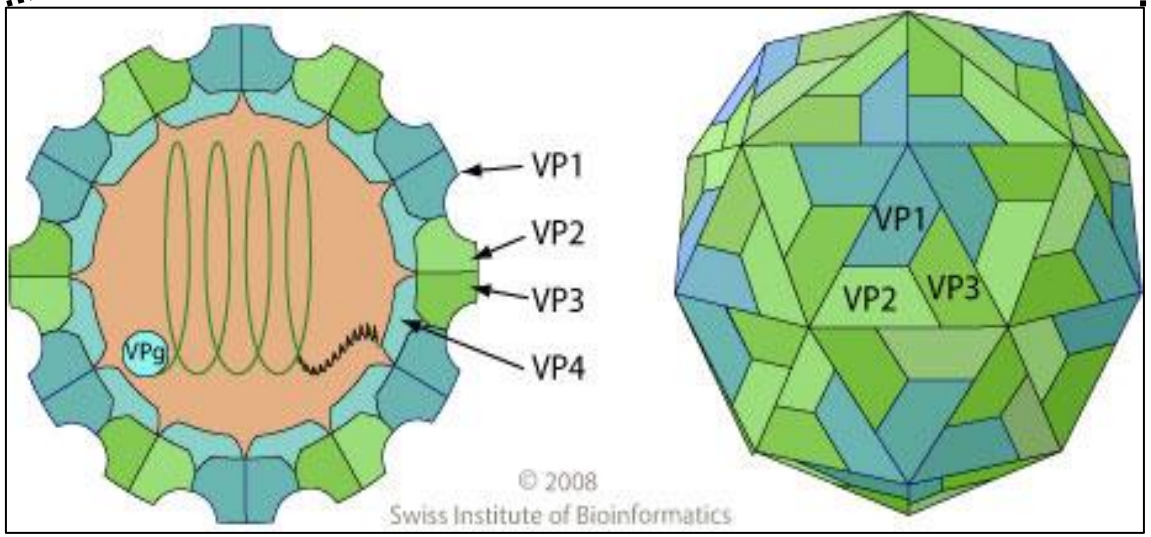
Complications

- Rare neurological complications
- Aseptic meningitis, brain stem encephalitis with neurogenic edema
- In infants and young children (mean age < 2 years old)
- **More commonly associated with EV-A71** (0.1-1.1% severe; 0.01-0.03% fatal)[1, 2]
- Long-term neurological sequelae[3]

1. Rev Med Virol 2019, 29: e2073.
2. eBiomedicine 2020, 62: 103078.
3. Eur J Paediatr Neurol 2018, 22:763-773.
4. Emerg Infect Dis. 2018, 24: 432-442.

Enteroviruses





- Family of *Picornaviridae*
- Genus *Enterovirus*
- Single-stranded positive sense RNA (~7.4 kb)
- capsid proteins VP1 – VP4
- VP1-3 receptor binding, antigenicity
- Non-structural polyprotein processing, replication
- Receptors- SCARB2, PSGL-1, heparan sulfate etc

Neurology Asia 2010, 16:1-15, https://viralzone.expasy.org/97.

Epidemiology & Immunological Considerations

Disease burden of HFMD

Known target population

Lessons from polio and current EV-A71 vaccines

Neutralizing antibody correlate of protection

Durability of immune protection

Immunogenicity requires complete capsid & is species specific

Disease Burden of HFMD

Table 2 Annual disability-adjusted life—year (DALY) losses in eight Asian countries/regions with 95% credible intervals (CI)

Country or region	DALY	95% CI
People's Republic of China (excluding Hong Kong and Taiwan)	75 881	(31 835 to 202 591)
Hong Kong special administrative region, People's Republic of China	285	(115 to 767)
Japan	5456	(2290 to 14 589)
Malaysia	2723	(1138 to 7281)
Singapore	259	(104 to 748)
Taiwan, Republic of China	1084	(435 to 3052)
Thailand	3928	(1644 to 10 536)
Vietnam	7248	(3042 to 19 414)

- **96 900 (95% CI 40 600 - 259 000) age-weighted DALYs per annum**

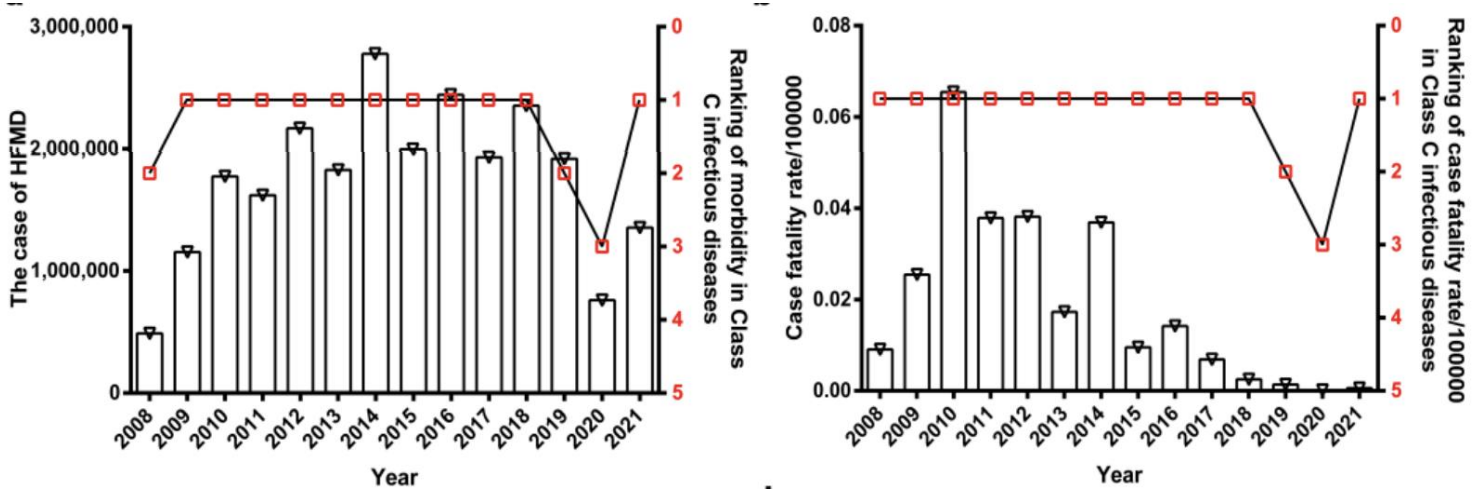
BMJ Global Health 2018;**3:**e000442.

Vietnam (2016–2017):

- **Total of 94,313 hospitalized HFMD cases**
- **HFMD economic burden- US$90,761,749**

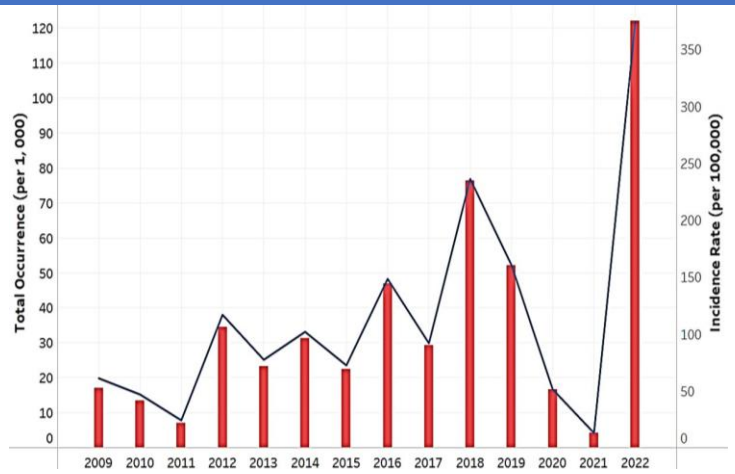
Open Forum Infectious Diseases 2019; 6: ofz284

China: HFMD morbidity cases remained at approximately 2 million



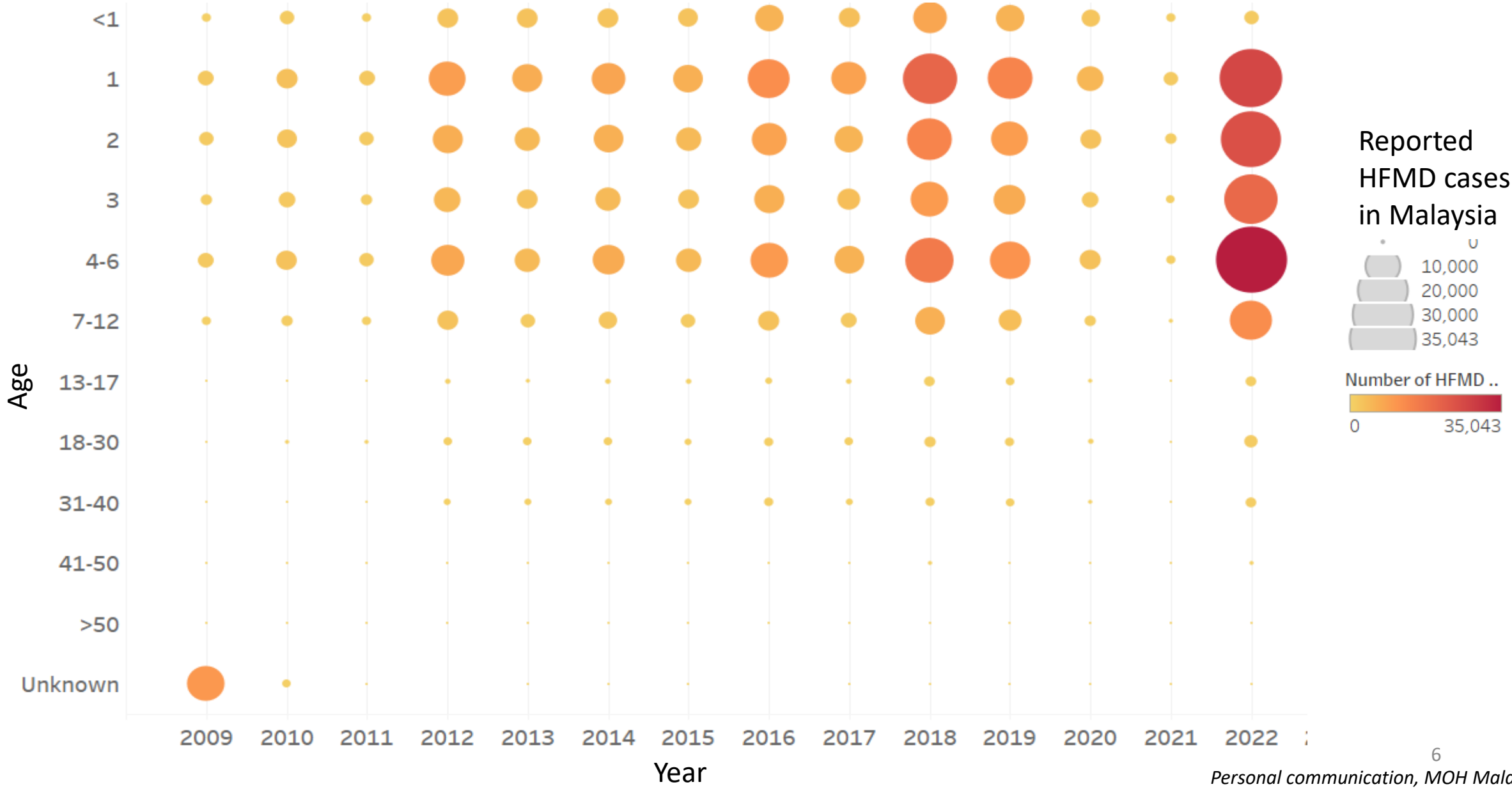
Int. J. Mol. Sci. 2023, 24:169.

Malaysia: Second most common infectious disease



Ministry of Health Malaysia.

HFMD affects young children



Personal communication, MOH Malaysia

EV-A71 Vaccines- Good Neutralizing Antibodies & Durable Immune Protection

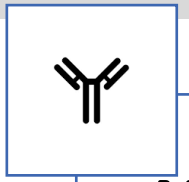
Organizations	Cell Line	Strain	Dosage (mg)	Population Target In trial	Remarks
Sinovac Biotech Co., Ltd (China)	Vero cell	C4a (H07 strain)	1	6–35 months old	94.8% efficacy Approved Dec 2015
Beijing Vigoo Biological Co., Ltd. (China)	Vero cell	C4a (FY7VP5 strain)	0.8	6–35 months old	97% efficacy Approved Dec 2016
CAMS (China)	KMB- 17 cell	C4a (FY- 23 K-B strain)	0.25	6–71 months old	94.7% efficacy Approved Dec 2015
NHRI (Taiwan)	Vero cell	B4	5 and 10	20–43 years adults	-
Enimmune Co. (Taiwan)	Vero cell	B4	0.25, 0.5, 1, 2 and 5	6 month –6 years old	-
Medigen Vaccine Biologics Co. (Taiwan)	Vero cell	B4	150	2 month - 6 years old	96.8% efficacy Approved 2023
Inviragen (Singapore)	Vero cell	B4	0.6 and 3	Adults	Terminated

Vaccine 2020, 38: 1-9.
https://www.who.int/docs/default-source/biologicals/ecbs/post-ecbs-who-ev71-vaccines-recommendations-final-3-nov-2020.pdf?sfvrsn=ce2ba6b5

Immune Responses (EV-A71)

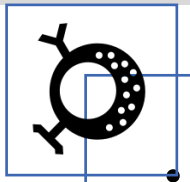
Innate

- Cytokine storm associated with IL-1 beta, IL-6, IL-10, IL-17A, MCP-1, IL-8 MIG, IP-10, G-CSF
- **activated by non-structural viral proteins**[1]



Humoral

- **Maternal antibodies** decline by 6 months
- IgM-detectable 3- 6 days after onset, **cross-reactive**
- 1:16-1:32 neutralizing antibody as immunological surrogate endpoint for EV71 vaccine protection[2]
- Seropositivity and seroconversion after two vaccine doses were **~100%** [3] **,>IU 36.2 IU/ml**[4]
- IVIG for treatment
- **dominant linear epitopes in mice and human are different**
- antibody-dependent enhancement only *in vitro* model

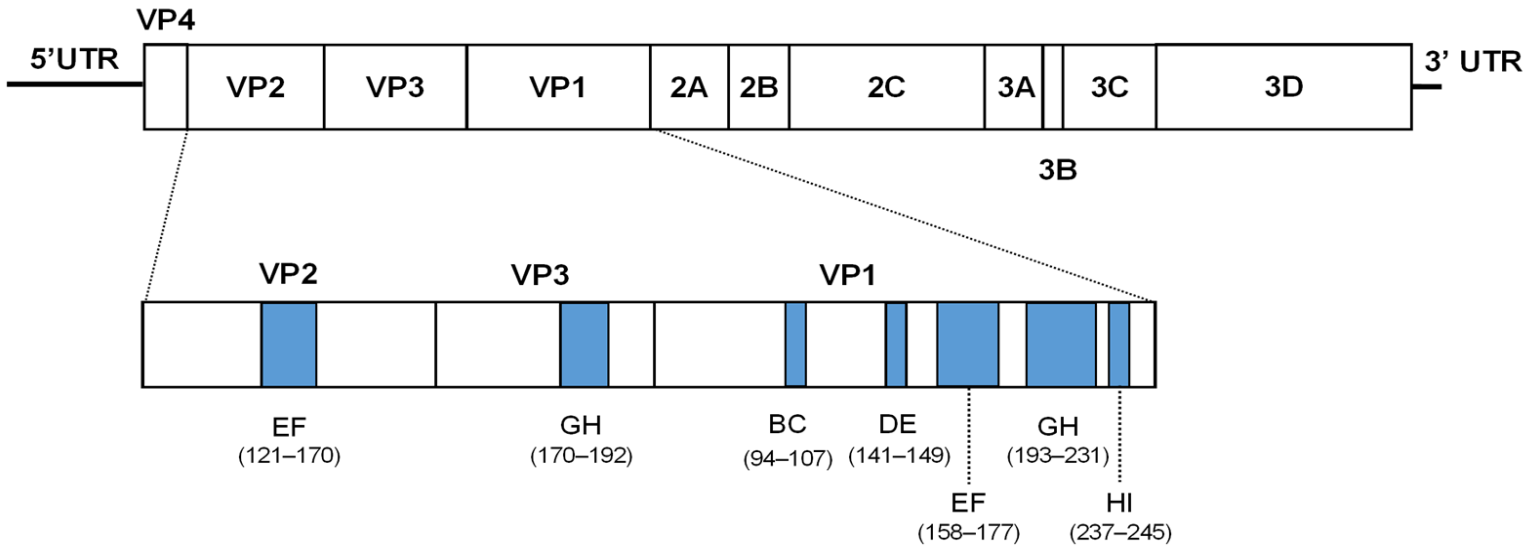


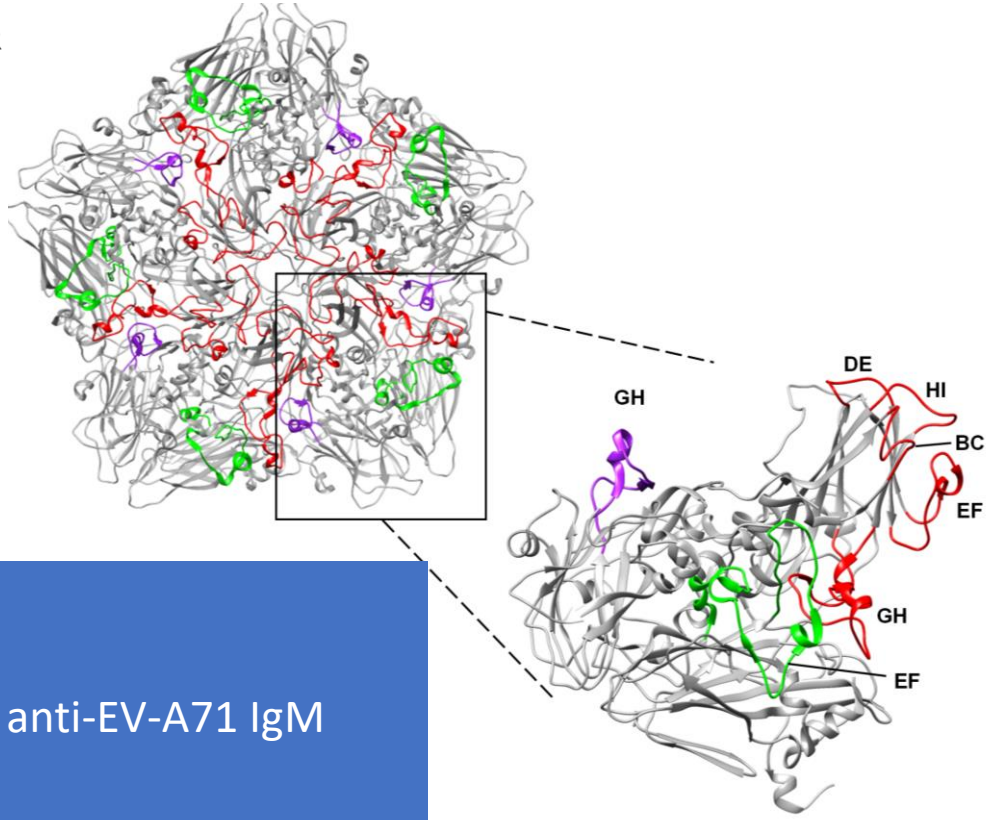
Cellular

- **Correlates with disease progression** and clinical outcome
- Th1 & pro-inflammatory cytokines in blood
- **Cross-reactive epitopes**[5]

Rev Med Virol 2019, 29: e2073.
1. Int. J. Mol. Sci. 2023, 24: 169.
2. Human Vaccine and Therapeutics 2022, 18: e2073751.
3 Lancet Reg Health West Pac 2021, 16: 100284.
4. Expert Rev Vaccines. 2017,16:945–949.
5. J Immunol 2013, 191: 1637–1647.

EV-A71 Antigenicity, Immunogenicity & Immune Protection



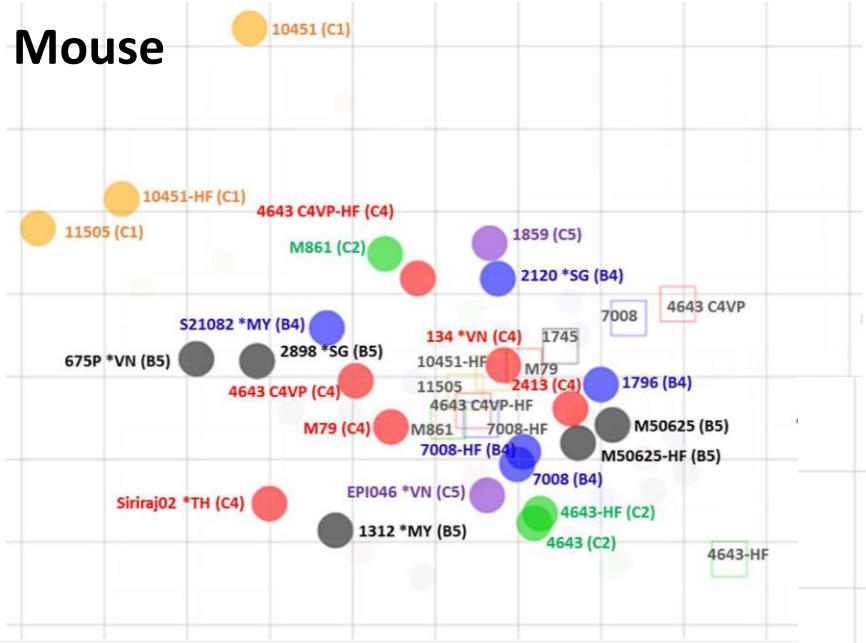


- Antigenic- **F-antigen** (D antigen in poliovirus)
- **Antigenic loops**- insertion, replacement
- The structural protein **VP1** is the main immunodominant site targeted by anti-EV-A71 IgM and IgG antibodies
- **DE loop- EV-A71 IgM**-specific immunodominant epitope
- Epitopes against **non-structural proteins** were also detected
- Antisera from **rabbits** injected with formalin-inactivated EV-A71 only recognized VP2-28 peptide (**aa 136–150 VP2**)
- **Mouse** antisera only recognized VP1-43 (**aa 211–225 VP1**= SP70)

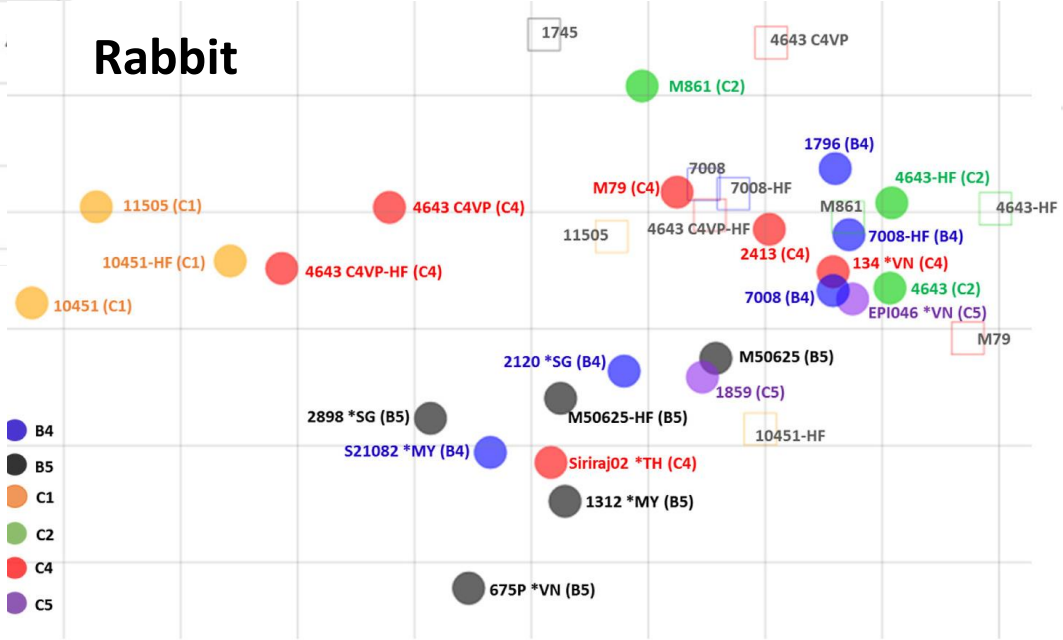
PLoS ONE 2016, 11: e0165659, Vaccines (Basel) 2023, *11*: 1363.

EV-A71 Antigenicity, Immunogenicity & Immune Protection

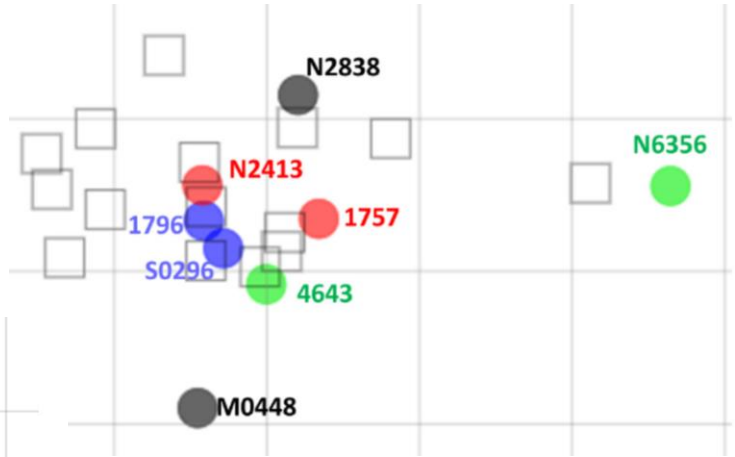
Mouse



Rabbit



Human



- **different but good/acceptable cross-reactivity between genogroups**
- **species-specific dominant epitopes**
- **careful interpretation of animal studies**

Antiviral research 2023, 212: 10559
Viruses. 2021, 13: 720.
Emerg Infect Dis 2016, 22:1562–1569
Vaccine 2009, 27: 3153–3158
PLoS Negl Trop Dis 2014, 8: e3044

Epidemiology & Immunological Considerations

Disease burden of other enteroviruses

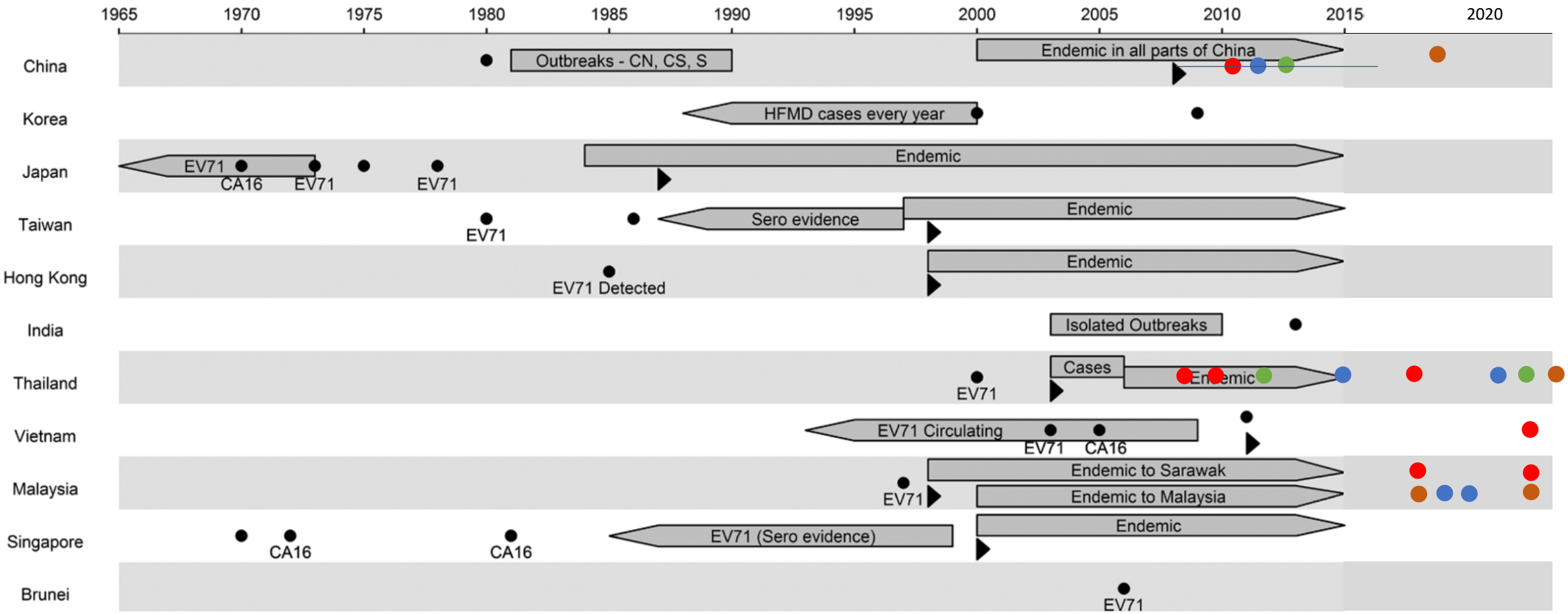
Antigenic diversity, Multiple serotypes and genotypes

Virulence & Recombination

Multivalent candidates

Immune Interference

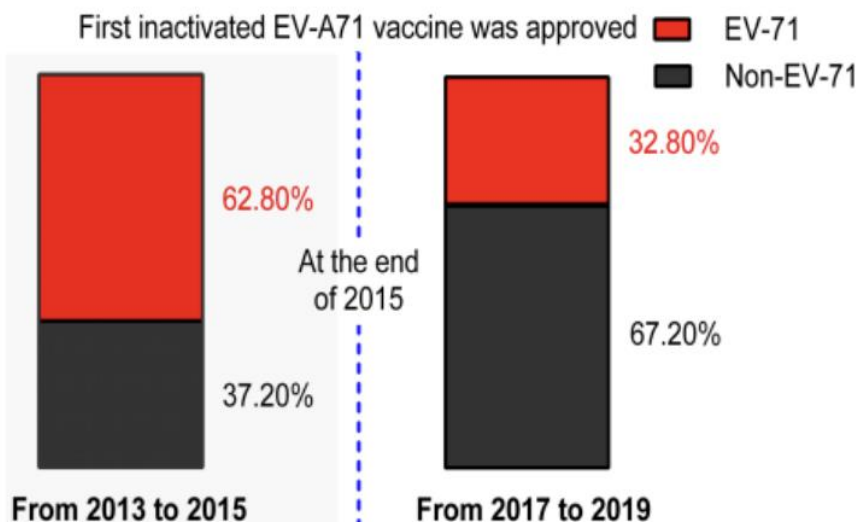
HFMD in Asia- Presence of Multiple Enteroviruses- The Need for Multivalent HFMD Vaccine

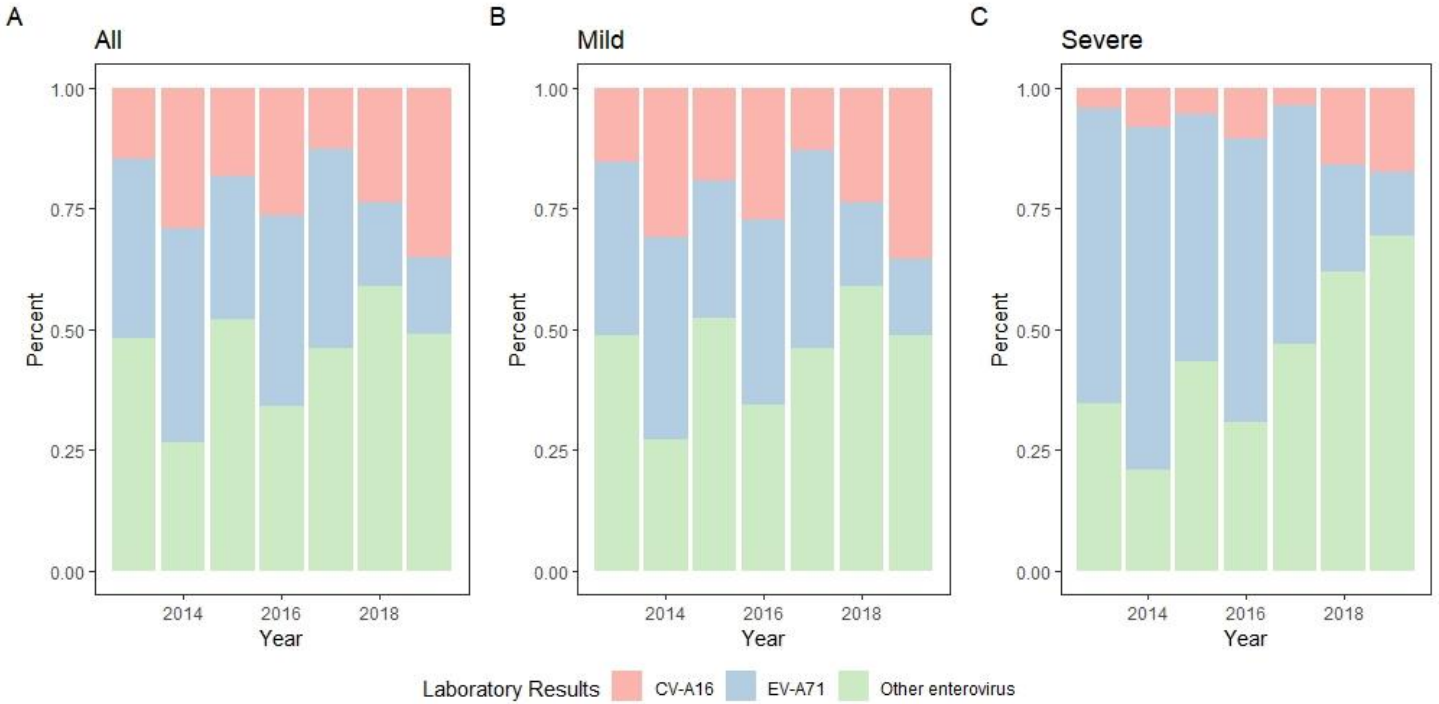


Modified from Pediatric Infectious Disease Journal (2016) 35: 285- 300
Virologica Sinica 2020, 35: 21-33, Biosafety and Health 2019, 1: 32-40
J Biomed Sci 2019, 26: 75, Viruses 2022, 15: 73.
Personal communication with National Public Health Laboratory, Malaysia

Characteristics of HFMD in Mainland China: Before and After Vaccine

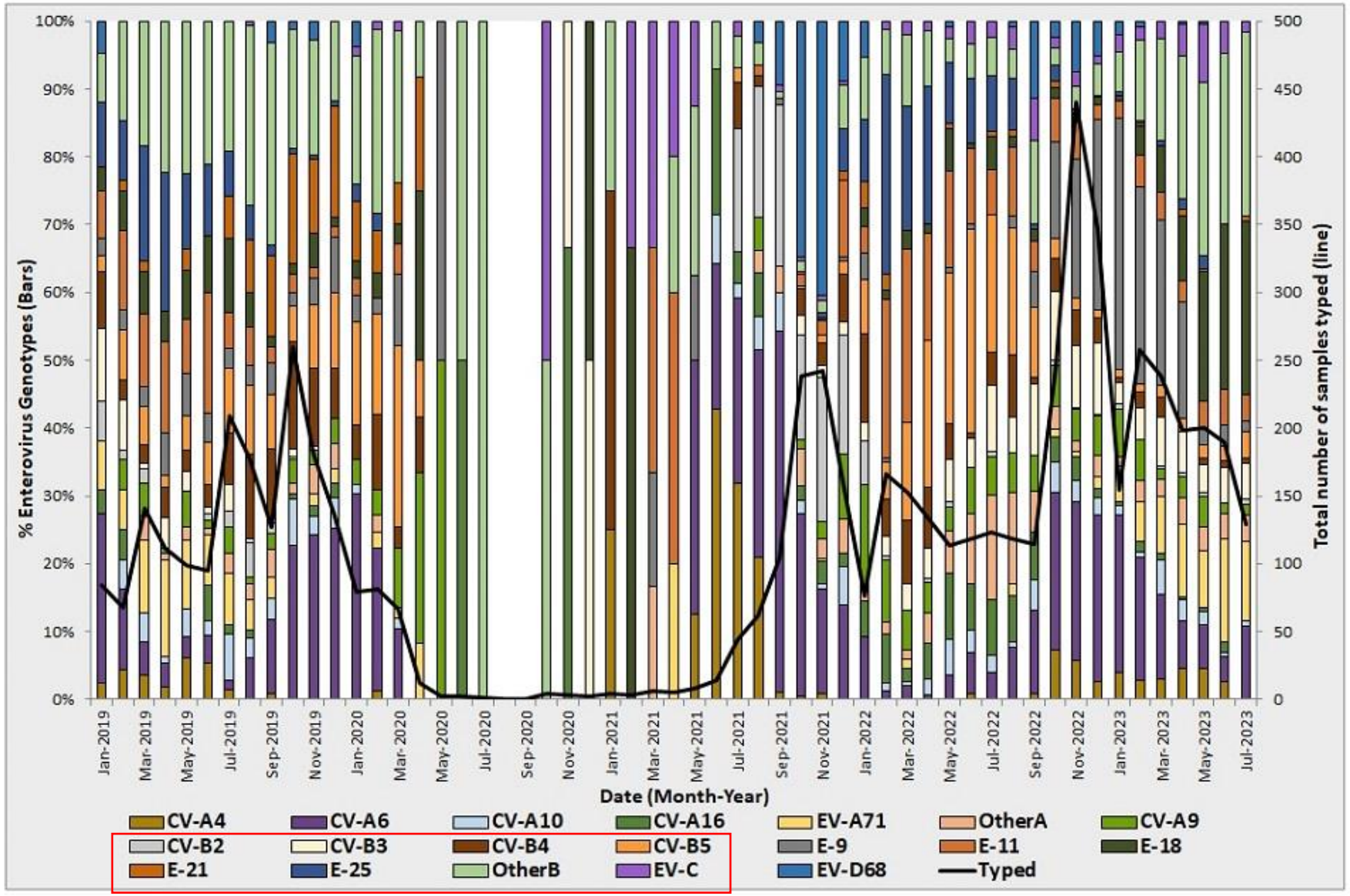
	2013-2015 (Before)	2017-2019 (After)	Change （%）
Incidence rates *	2800.78	2575.29	-8.05
Severe illness rates *	18.97	7.17	-62.20
Mortality rates *	0.37	0.06	-83.78
Severe /Cases （%）	0.68	0.28	-58.82
Death/Cases （%）	0.01	0.00	-100.00
Death/Severe Cases （%）	1.97	0.85	-56.85





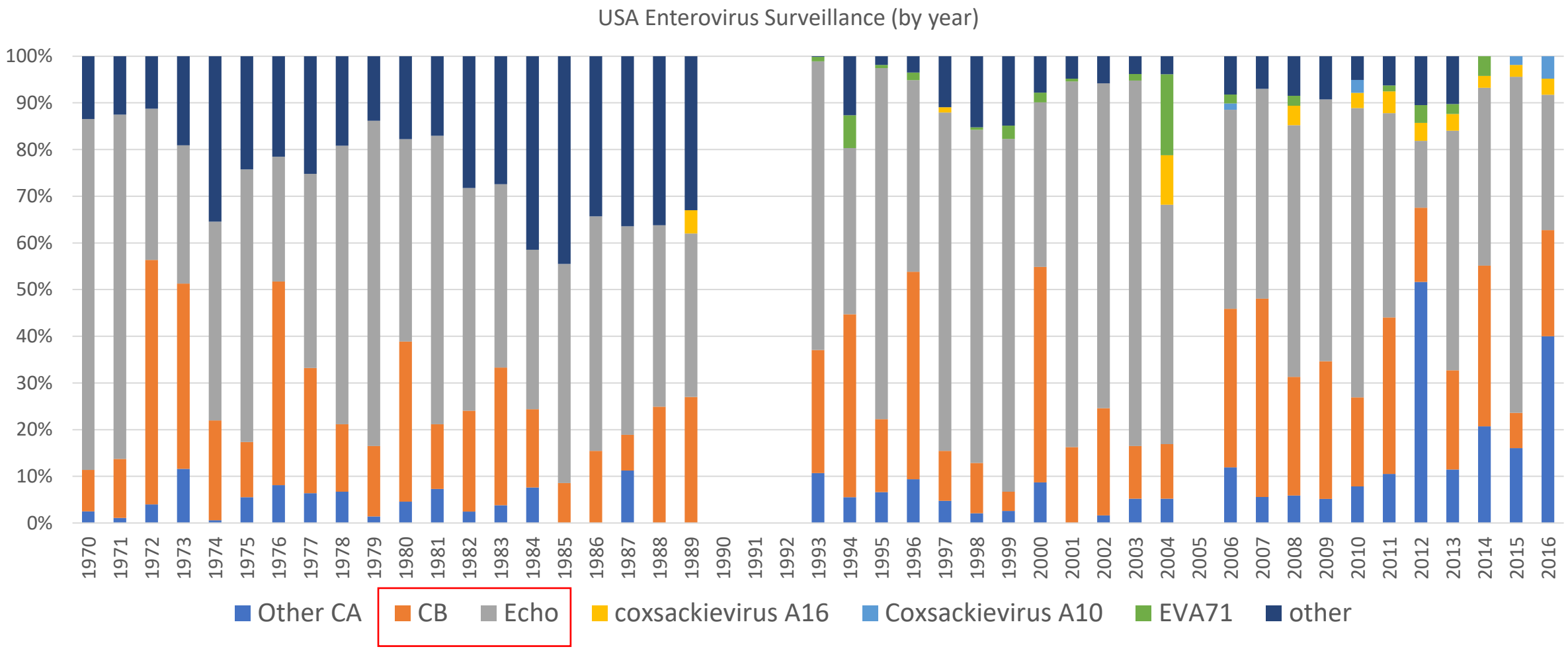
The Lancet Regional Health - Western Pacific 2022;20: 100370, Vaccine 2021, 39: 3319–3323

Circulation of Other enteroviruses-UK



https://www.gov.uk/government/publications/enterovirus-strain-characterisation/enterovirus-summary-of-strain-characterisation

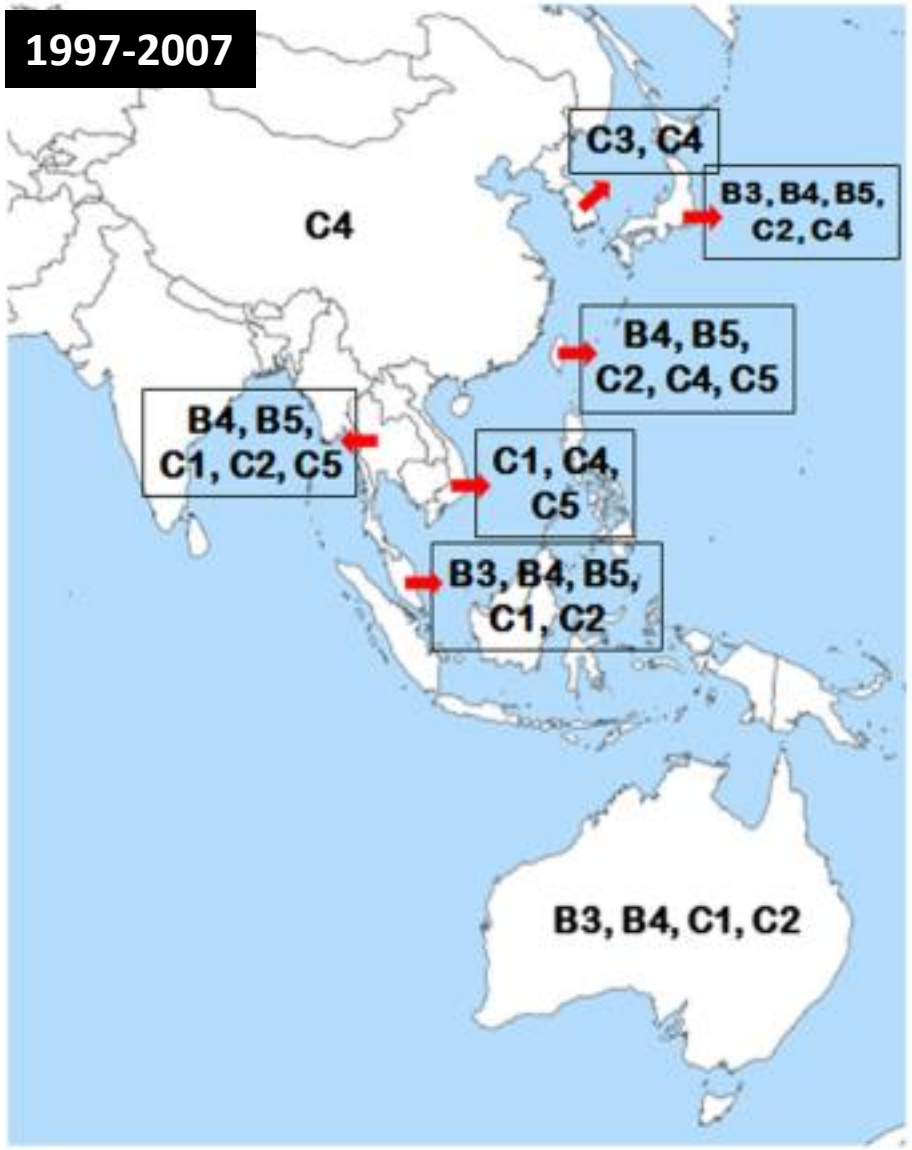
Circulation of Other Enteroviruses-USA

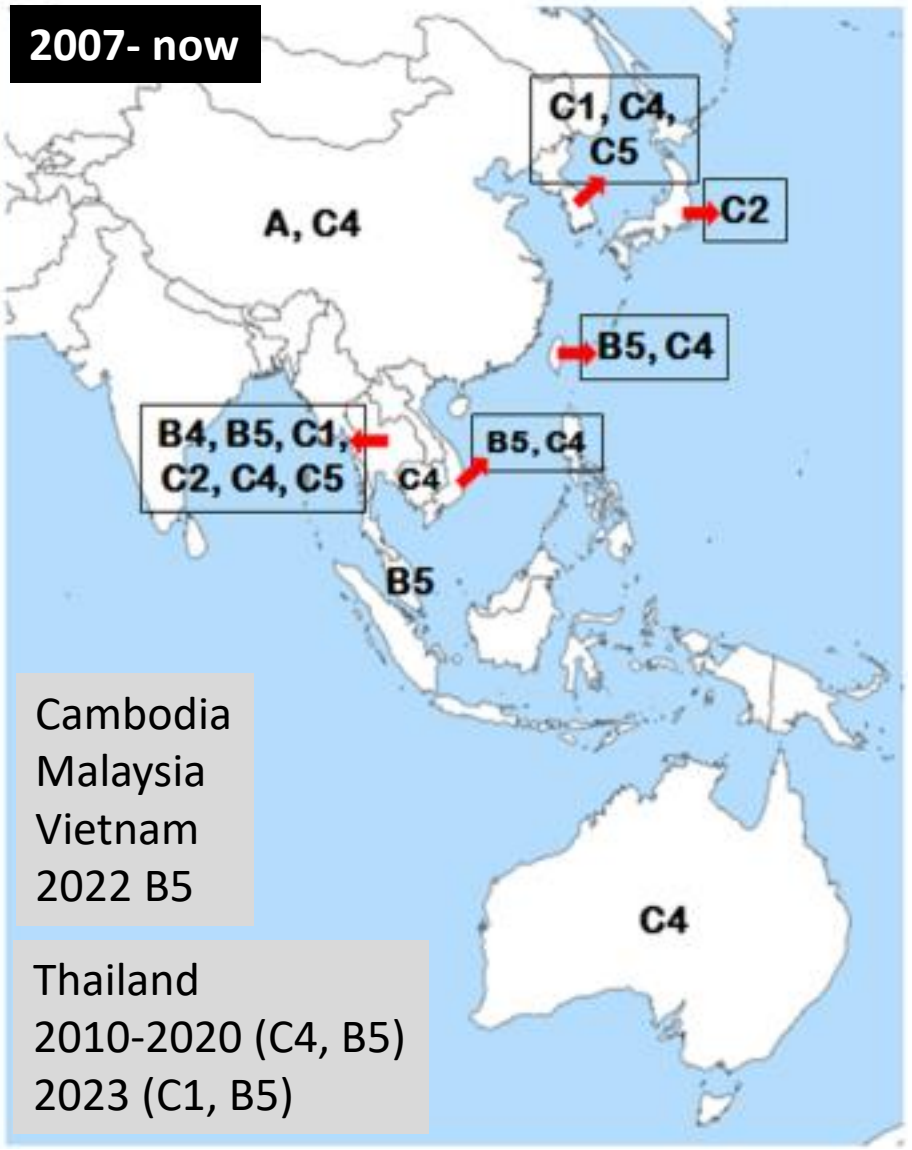


Do we need to add other vaccine candidates?

Data from CDC https://www.cdc.gov/non-polio-enterovirus/resources.html

Monitoring virulence & recombination in EV-A71





Cambodia
Malaysia
Vietnam
2022 B5

Thailand
2010-2020 (C4, B5)
2023 (C1, B5)

- Severe HFMD Vietnam- C4[1], less severe Malaysia- B4[2]
- Virulence in SCARB2-transgenic mice clade B5 (I, II), C4 (II-IV)[3]
- Virulent mutations VP1 145, 244[4]
- Recombination alters fitness and virulence[5]

J Biomed Sci 2019, 26: 75; personal communication APNES 2023

1. Eurosurveillance 2018, 23: 1800590
2. Clinical Infectious Diseases 2007; 44: 646-656
3. J Virol 2021, 95: e0151521
4. PLoS Pathogens 2019, 15: e1007863
5. Emerg Microbes Infect 2021, 10: 713-724.

Learn from Multivalent HFMD Vaccines

TYPES

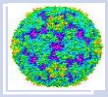
- Inactivated
- Live-attenuated
- Subunit- peptide, VP1 etc
- Virus-like particles
- Bivalent/ Trivalent/ Tetravalent/ Hexavalent

CHARACTERISTICS

- HFMD disease burden: EV-A71, CV-A6, CV-A10, CV-A16
- HFMD and other enteroviruses: CV-B, Echo
- Innate and cellular responses- non-structural viral proteins
- Cross protection- conserved regions
- Balance Immune interference

Int. J. Mol. Sci. 2023, 24: 169.
Therapeutic Advances in Vaccines and Immunotherapy 2019;7.
Hum Vaccin Immunother 2015, 11:2688-704
Vaccines 2014, 32: 6177-6182.

Epidemic Dynamics of Enterovirus Serotypes and Implications for Vaccination (Immune interference?)



Infection with EV-A71 or CV-A16 serotype provide transient immunity against each other

Vaccination- Serotype replacement by CV-A16 transient, big reduction in the burden of EV-A71-associated HFMD

A mass EV-A71 vaccination program of infants and young children will reduce HFMD burden.

Possible asymmetry in its strength such that CV-A16 serves as a stronger forcing on EV-A71.

PLoS Med 201, 13: e1001958, J. R. Soc. Interface 2018, 15: 20180507.

Summary: Epidemiology & Immunological Considerations

STRENGTHS

- Disease burden of HFMD
- Known target population
- Lessons from polio and current EV-A71 vaccines
- Neutralizing antibody correlate of protection
- Durability of immune protection
- Immunogenicity requires complete capsid & is species specific

FUTURE IMPROVEMENTS

- Disease burden of other enteroviruses
- Antigenic diversity, Multiple serotypes and genotypes
- Virulence & Recombination
- Multivalent
- Immune Interference