

# **Enterovirus 71 (EV71)**

## **Fondation Merieux Conference - Asia Pacific Vaccinology 2015**

**Bangkok, Thailand**

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**1 Research Link**

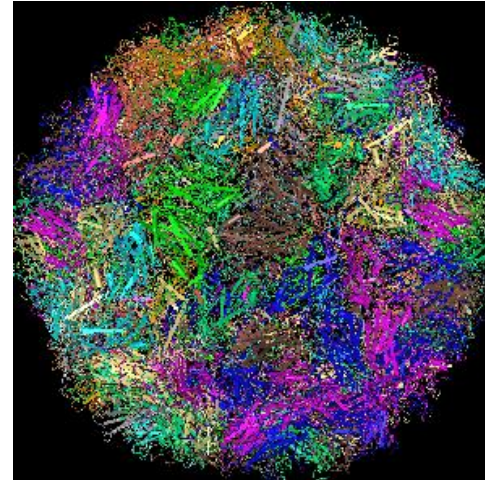
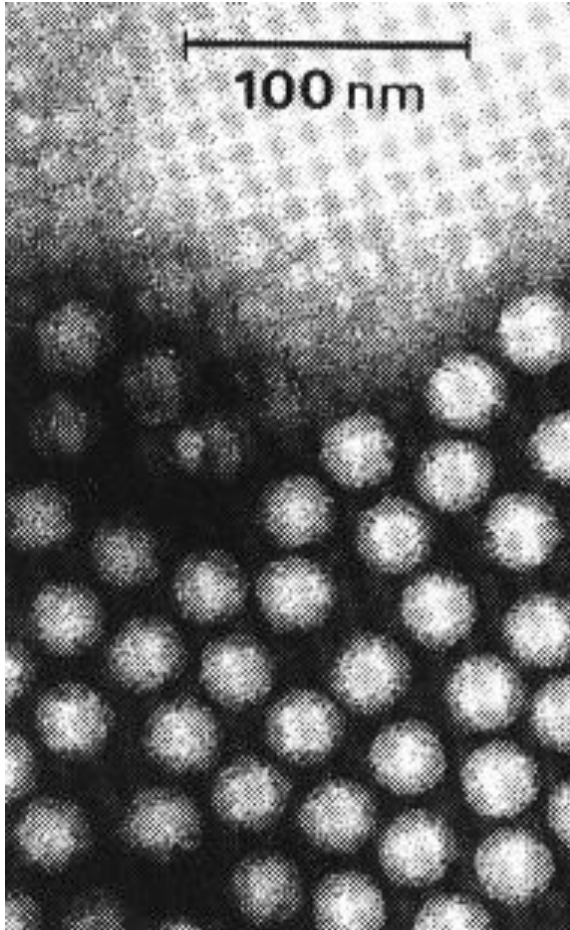
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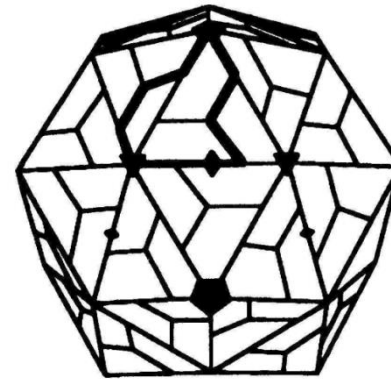
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# Enterovirus 71 (EV71)

Electron Micrograph

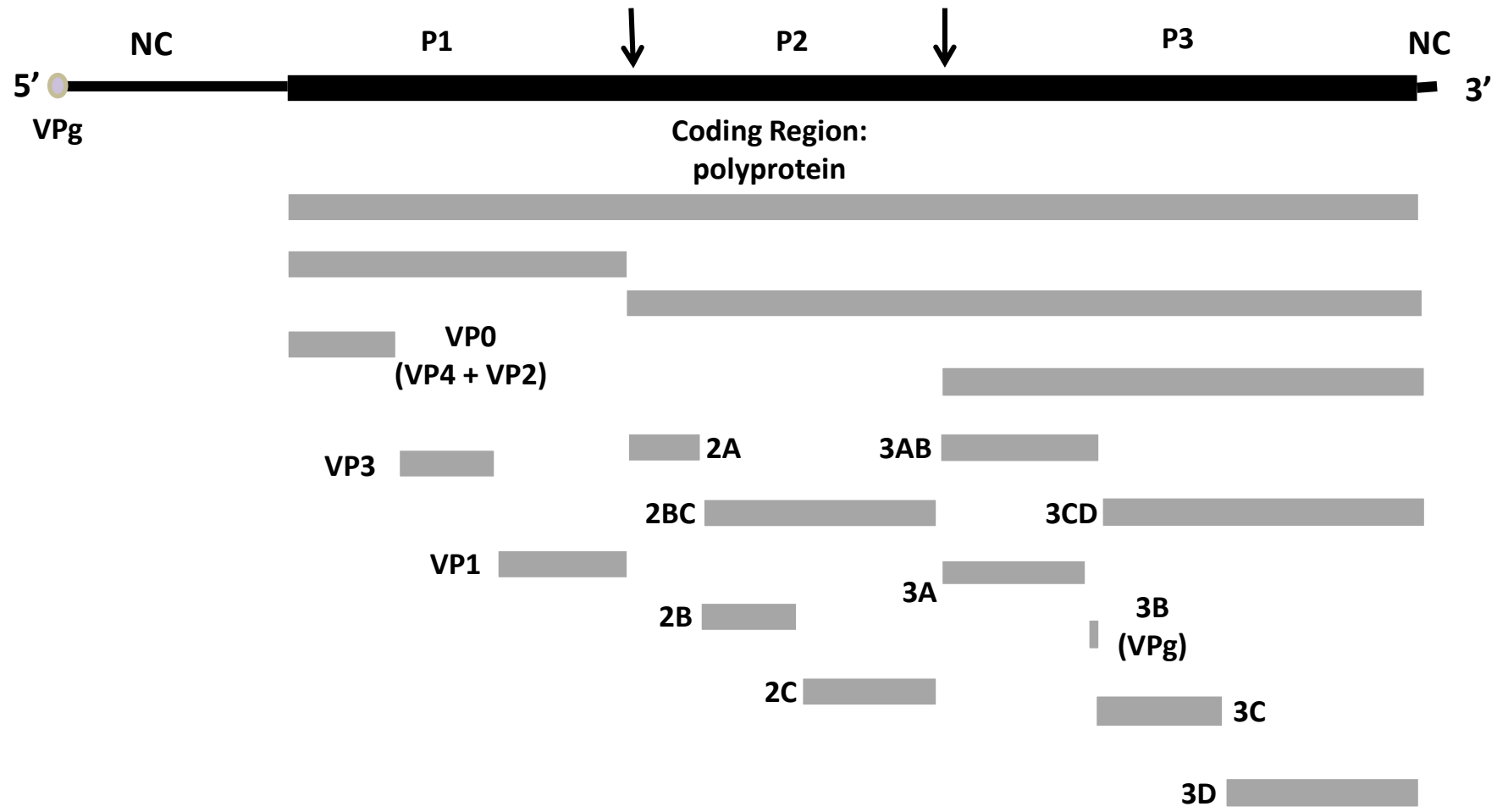


Crystal Structure



Schematic Diagram

# Genome Structure and Encoded Proteins of EV71







# Disease and Public Health Burden due to EV71

An important emerging viral disease of children.

## Besides causing HFMD.

- Non-specific febrile illness
- “Common cold”
- Exanthema
- Herpangina
- Acute pharyngitis
- Infantile pneumonitis
- Infantile diarrhoea
- Aseptic meningitis
- **Acute flaccid paralysis**
- **Acute Encephalitis**
- **Acute encephalomyelitis**  
**+/- pulmonary oedema**

Outbreaks of HFMD with neuro-invasion and fatalities.

## Asia-Pacific region:

**1997, 2000, 2003, 2006 - Malaysia**

**1998, 2000/2001 - Taiwan**

**1999 - Perth Australia**

## China:

2008: 488,955 HFMD, 126 Fatalities.

2009: 1,155,525 HFMD, 353 Fatalities.

2010: 1.7 million HFMD, 905 Fatalities.

# Disease Manifestations of EV71

## Dermatophilic (Coxsackievirus A16-like):

- **HFMD**
- **Exanthemata**
- **Enanthem - Herpangina**

## Neurotrophic (Poliovirus-like):

- **Neck Stiffness (Meninges)**
- **Acute Paresis/Flaccid Paralysis (Pyramidal nervous system)**
- **Ataxia, Tremor (Extra-Pyramidal nervous system)**
- **Acute neurogenic pulmonary oedema (Autonomic system)**

# Issues, Challenges and Solutions

**Animal Model of EV71 Neuro-Infection,  
Disease and Pathology**

**EV71 Vaccine**



# What are the Current Published Animal Models?

## **Monkey Models: *Macaca fascicularis*, Rhesus**

### **Intra-spinal Inoculation:**

**Hashimoto and Hagiwara.**

**Acta Neuropathologica 1982; 58: 125-132.**

### **Intravenous Inoculation:**

**Nagata N, et al.**

**J Med Virol 2002; 67: 207-216.**

**Arita M, et al.**

**J Gen Virol 2005; 86: 1391-1401.**

### **Intracerebral, Intravenous, Respiratory and Oral Routes:**

**Zhang Y, et al.**

**Laboratory Investigation 2011; 91: 1337-1350.**



# What are the Current Published Animal Models?

## Small Animals: Mouse Models

Immuno-competent mice (**BALB/c, C3H/HeN, ICR**): Intracranial, Intravenous, Intraperitoneal, Oral.

Yu CK, et al. *J Biomed Sci* 2000; 7: 523-6.

Chua BH, et al. *J Gen Virol* 2008; 89: 1622-32.

Khong WX, et al. *J Virol* 2012; 86: 2121-31.

Wang YF, et. Al. *J Biomed Sci* 2014; doi:10.1186/1423-0127-21-31.

(Age < 14 days, myositis, need > 10<sup>7</sup> pfu/ml)

Immuno-compromised mice:

**NOD/SCID, A129, AG129 mice.**

Transgenic Mice (**PSGL-1, SCARB2**).

Liu J, et al. *Arch Virol* 2012; 157: 539-43.

Lin YW, et al. *PLoS One* 2013, : 8:e57591.

Fujii K, et al. *PNAS* 2013; 110: 14753-8.

Mouse-adaptation in newborn mice:

**Intracerebral adaptation.**

**Intra-skeletal muscle adaptation.**

Mouse-adaptation in immuno-compromised mice.

# Current Published Animal Models

- Lack “Face Validity” (i.e., lack full resemblance to human symptoms). **(Fulminant Pulmonary Edema)**
- Lack “Construct Validity” (i.e., lack similarity to the underlying cause of the human disease). **(Myositis)**

None of the EV71 strains is able to replicate in in-vitro culture mouse cells

## TLL mouse model of EV71 infection

**EV71:TLLm and EV71:TLLmv** - Strains that are able to replicate efficiently in in-vitro culture mouse cells.



# Public Health Perspective

- **Urgent Need for an Effective and Efficacious Vaccine to Control and Prevent Hand Foot and Mouth Diseases and associated neurological complications.**

## **EV71 Vaccines Under Research and Development**

- **cDNA vaccine.**
- **Subunit vaccine (VP1 peptide).**
- **Virus-like particle (VLP)**
- **Inactivated enterovirus 71.**
- **Live attenuated enterovirus 71.**



# **Development of Injectable Inactivated EV71 vaccines**

**(Based on Salk Inactivated Poliovirus Vaccine Model)**

# Inactivated EV71 Vaccine Development

**1975 Bulgaria, Europe.**

**Febrile illness: 705 reported cases.**

**545 (77.3%) cases of aseptic meningitis**

**149 (21.1%) cases of paralytic disease.**

**44 fatalities.**

**(Chumakov et al., Arch Virol 1979; 60: 329-40).**

# Disease Burden

## Outbreak of HFMD and Fatalities in China

**2008: China CDC & Office of WHO in China**

**Cases of HFMD: 488,955**

**Fatalities: 126**

**2009: Yang et al. Virology J 2011; 8:508.**

**Cases of HFMD: 1,155,525**

**Fatalities: 353**

**2010: Zeng et al. J Clin Virol 2012; 53(4): 285-9.**

**Cases of HFMD: 1.7 million**

**Severe neurological complication: 27,000**

**Fatalities: 905**

# Development of Injectable Inactivated EV71 vaccines

(based on Salk Inactivated Poliovirus Vaccine Model)

**Parental (Injectable) Inactivated EV71 vaccines at various stages of development and clinical trails:**

- **Beijing Vigoo, China National Biotech Group (China), EV71-C4 strain.**
- **Sinovac (China), EV71-C4 strain.**
- **Institute of Medical Biology (CAMS) (China), EV71-C4 strain.**
- **National Health Research Institute (Taiwan), EV71-B4 strain**
- **Inviragen (Singapore), EV71-B2 strain.**



# Phase III Clinical Trials (Summary)

Vaccine Producer	Beijing Vigoo Biological	Sinovac Biotech	Institute of Medical Biology, CAMS
Total Population	10,245	10,007	12,000
Age (month)	6 - 35	6 - 35	6 - 71
Dosing (unit –U)	320	400	100
Immunization Schedule (day)	0, 28	0, 28	0, 28
Adjuvant	Alum	Alum	Alum
Efficacy (against EV71-associated HFMD)	90.0%	94.8%	97.3% - 97.4%

# **Development of Live Attenuated Oral EV71 Vaccine (Based on Oral SABIN Poliovirus Vaccine Model)**

# Development of Live Attenuated Oral EV71 Vaccine (Based on Oral SABIN Poliovirus Vaccine Model)

Hashimoto I. and Hagiwara A.

*J. gen. Virol.* (1982), **64**, 499–502. *Printed in Great Britain*

499

Key words: *enterovirus/virulence/temperature-sensitive mutant/fingerprinting*

## Isolation of a Temperature-sensitive Strain of Enterovirus 71 with Reduced Neurovirulence for Monkeys

JOURNAL OF VIROLOGY, Sept. 2007, p. 9386–9395

Vol. 81, No. 17

0022-5382/07/\$08.00+0 doi:10.1128/JVI.02856-06

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### An Attenuated Strain of Enterovirus 71 Belonging to Genotype A Showed a Broad Spectrum of Antigenicity with Attenuated Neurovirulence in Cynomolgus Monkeys<sup>†</sup>

Minetaro Arita,<sup>1\*</sup> Noriyo Nagata,<sup>2</sup> Naoko Iwata,<sup>2</sup> Yasushi Ami,<sup>3</sup> Yuriko Suzaki,<sup>3</sup> Katsumi Mizuta,<sup>4</sup> Takuya Iwasaki,<sup>5</sup> Tetsutaro Sata,<sup>2</sup> Takaji Wakita,<sup>1</sup> and Hiroyuki Shimizu<sup>1</sup>

# Development of Oral Combined Live Attenuated Vaccine against HFMD

**“18 Years (1998 – 2015)  
JOURNEY”**



# HFMD in Malaysia

**May 1997; Sibu, Sarawak.**

**July 1997; FT, Peninsular Malaysia.**

**Number of cases of HFMD?**

**41 deaths reported (29 Sarawak, 12 Peninsular Malaysia).**

**Virus: EV71, CA16.**

# HFMD in Taiwan (1998)

129,106 reported cases (estimated 10% of total cases).

405 hospitalised for severe disease (80%  $\leq$  5 years old).

78 fatalities.

Serious complications: Encephalitis, Encephalitis and pulmonary oedema or haemorrhage, Aseptic meningitis, Myocarditis with encephalitis, Acute flaccid paralysis.

65 (83%) died of Encephalitis with pulmonary oedema or haemorrhage.

# Challenges of EV71 Vaccine

## Challenges inherent in the viruses causing HFMD:

- HFMD due to a number of enteroviruses, especially CA16 and EV71.
- Genetic and Antigenic Diversity of EV71.
- EV71 undergoes “rapid” Genetic Mutation and Antigenic Drift in the last 16 years.

## Challenges inherent in inactivated vaccine:

- Need for parental administration.
- High cost.
- Need for adjuvant with its associated side-effects.
- Reduced “Robustness/Quality” of neutralizing antibodies due to structural changes during the process of inactivation.
- Do not induce gut immunity. Thus, do not prevent silent EV71 infections in intestinal mucosa that lead to transient carriers and shedding of the virus.
- Public Health Perspective - ? Feasibility of Mass Vaccination

# Development of Live Attenuated Oral EV71 Vaccine

(Based on Oral SABIN Poliovirus Vaccine Model)

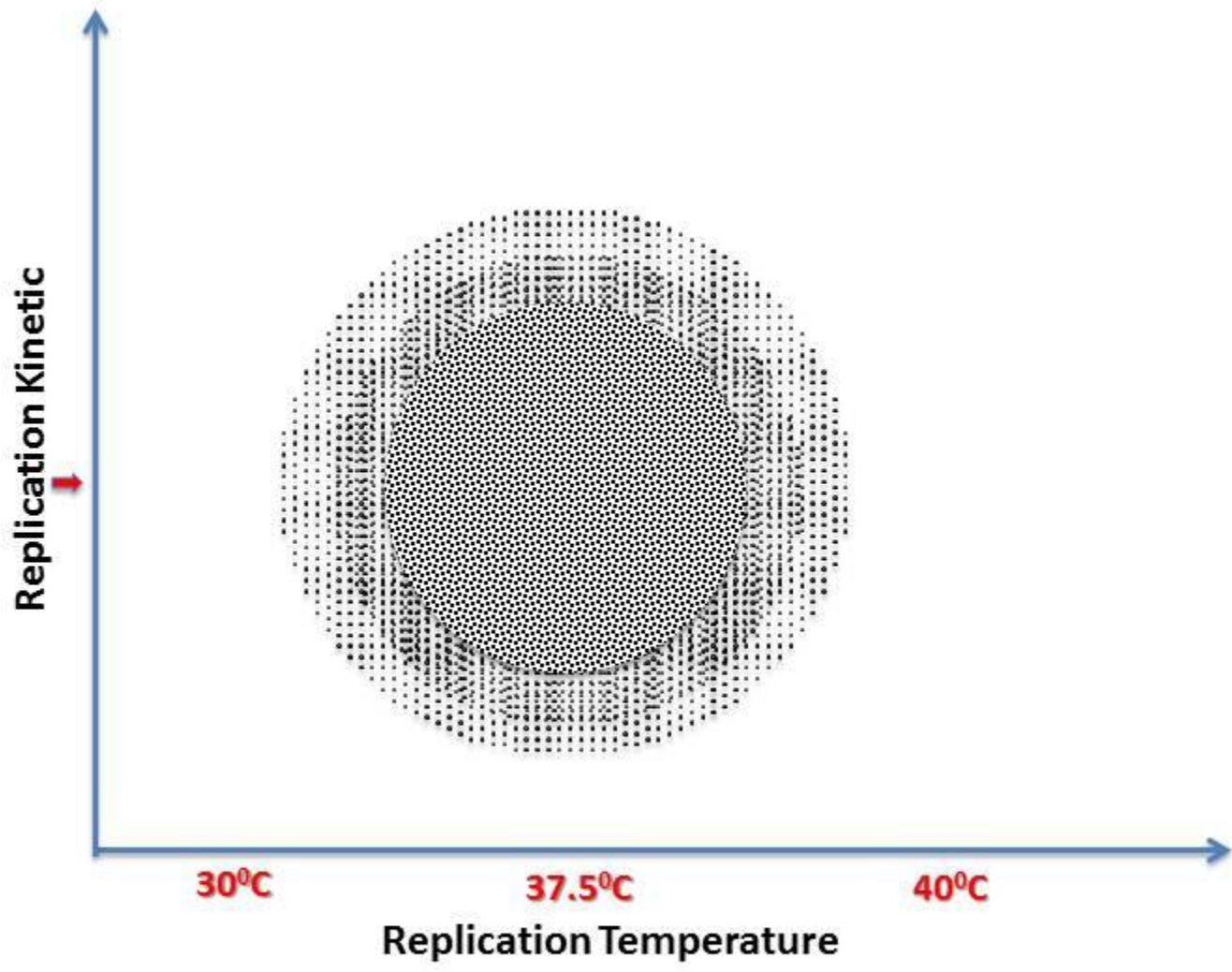
## Phenotypic and Genetic **STABILITY and REVERSION**

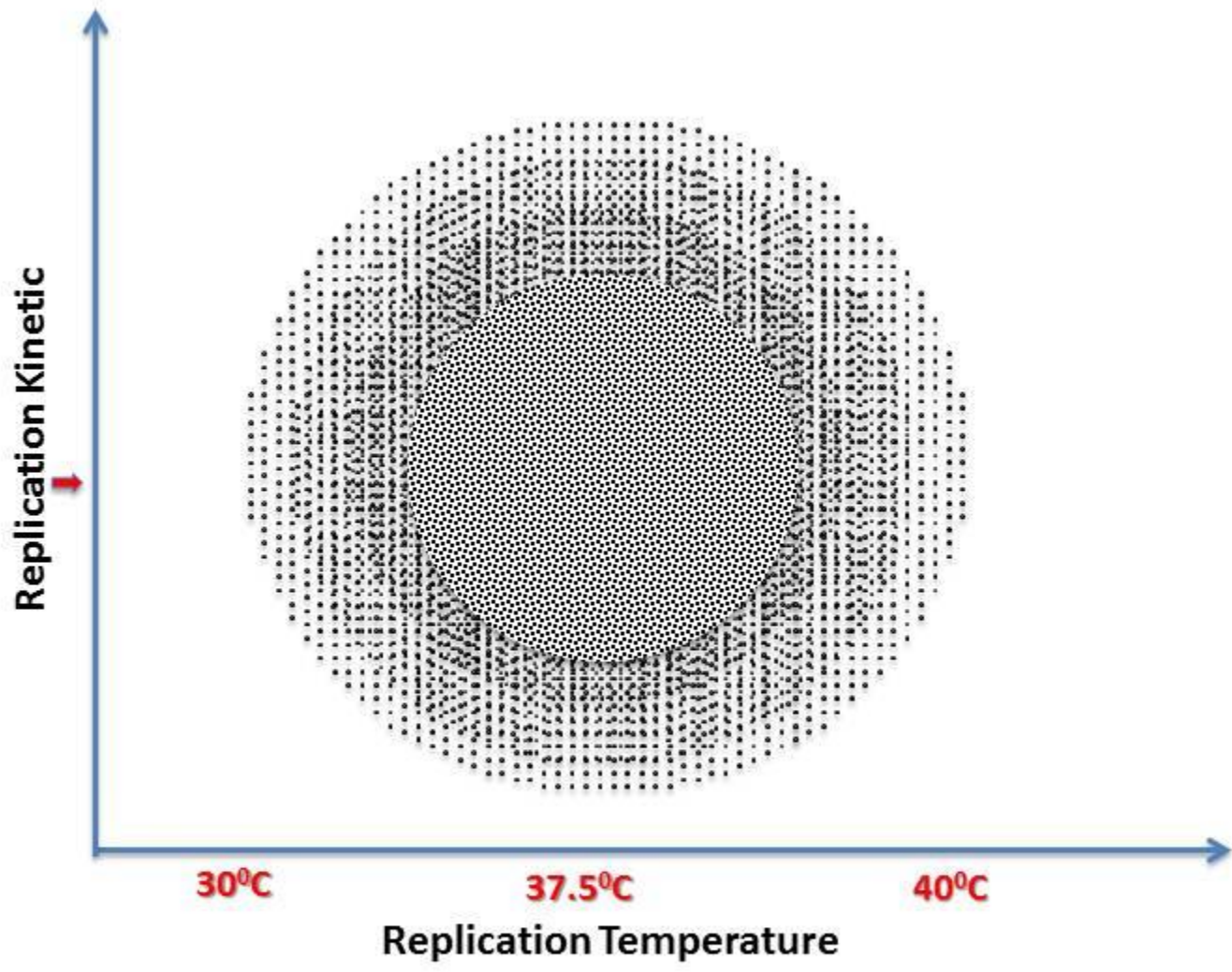


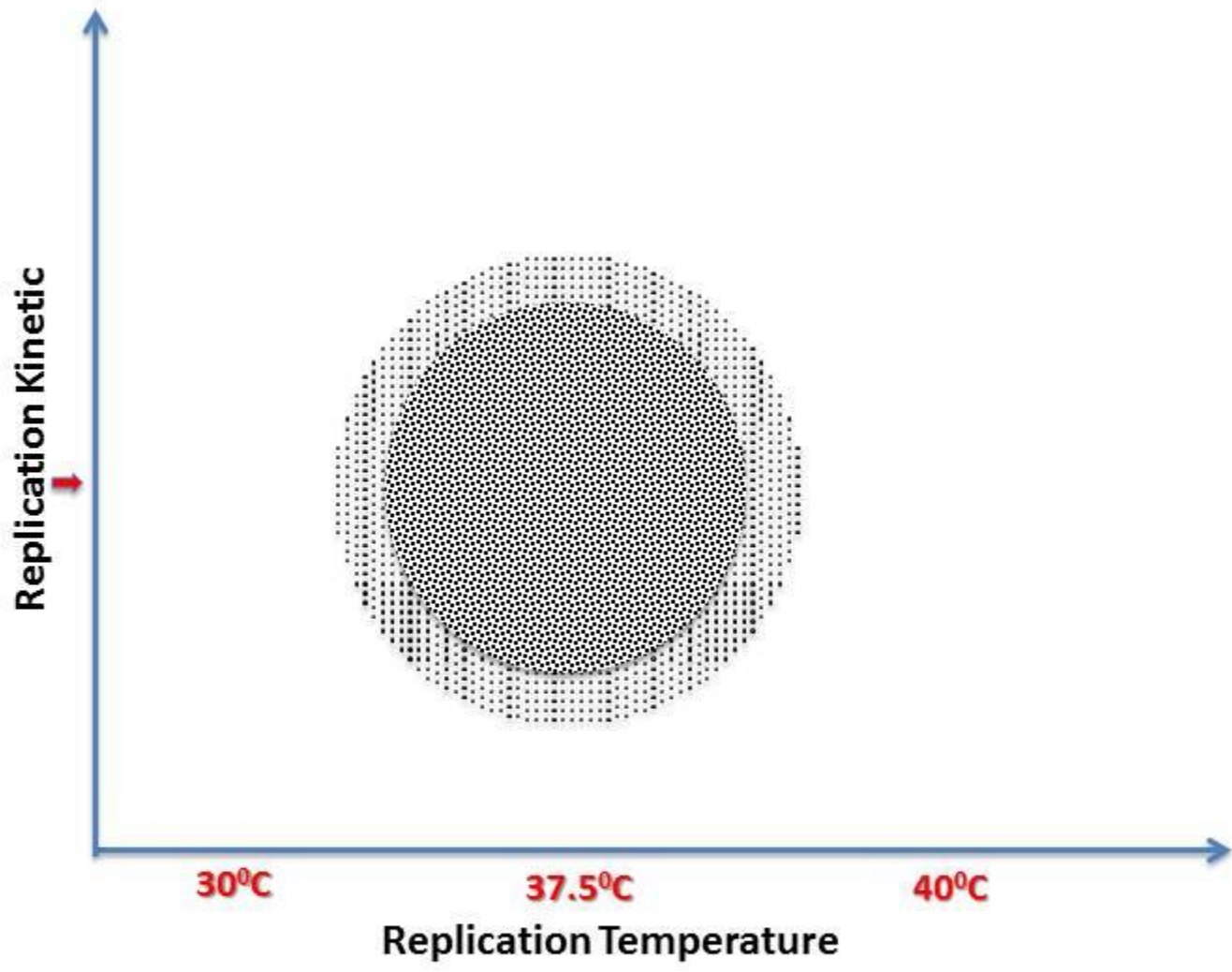
Searching for the  
**STABLE TEMPERATURE SENSITIVE**  
**EV71 Strain**

# Mutation Rates

**$3 \times 10^{-3}$  to  $2 \times 10^{-6}$  ( $1.6 \times 10^{-4}$ )**







# Enterovirus Entry, Replication and Morphogenesis

## Genome Replication:

**5'NC – “cloverleaf”, 3'NC**

**2BC, 2B, 2C; 3AB, 3A, 3B; 3CD, 3C, 3D**

Host cellular protein (Reticulon 3)

## Translation (Viral Protein Synthesis):

**5'NC-IRES, 2A;**

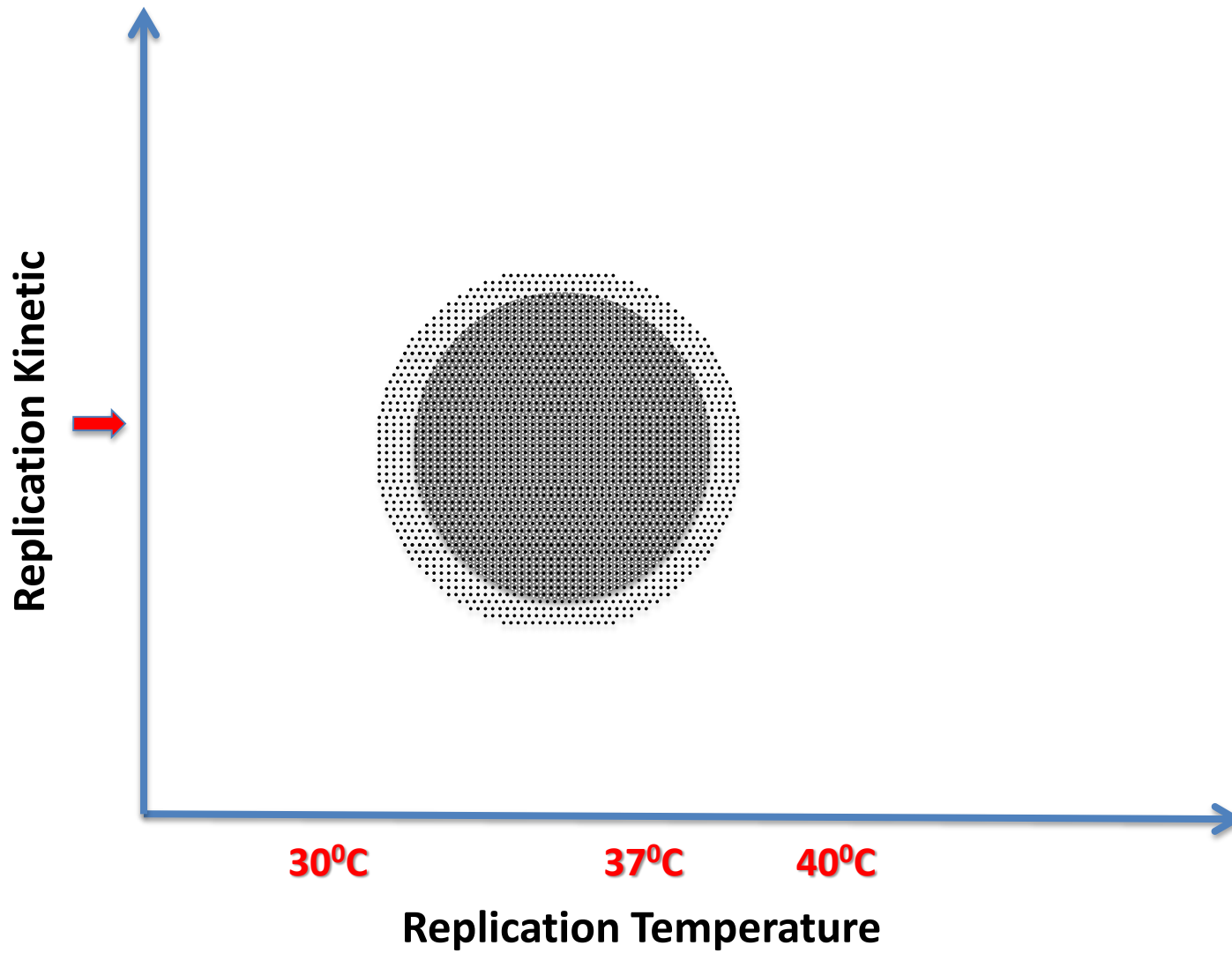
host cellular protein initiation complex, host cellular ribosomes

## Attachment, Uncoating and Assembly:

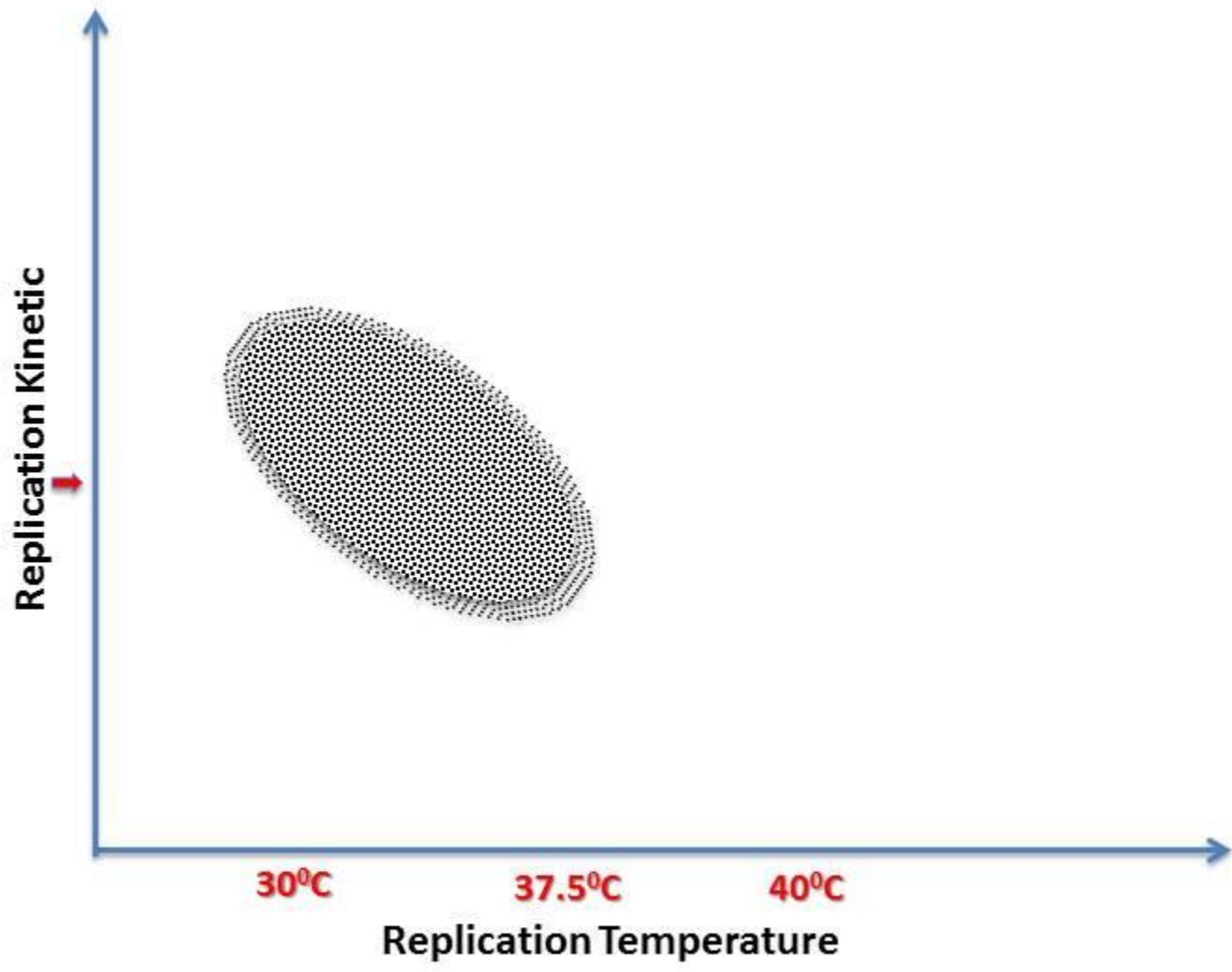
**VP1, VP2, VP3; VP1, VP2, VP3, VP4;**

**VP0, VP1, VP3, VPg, 2C, RNA**





**Candidate Attenuated EV71 strains:  
EV71:TLL $\alpha$ , EV71:TLL $\beta$  or EV71:TLL $\gamma$  strains**



**Candidate Attenuated EV71 strains:  
EV71:TLL $\alpha$  or EV71:TLL $\beta$  strains**

# Phenotypic Stability

# Genetic Stability

# EV71:TLL $\beta$ P20

**[Stable Cold-Adapted Temperature-Sensitive/Conditional Lethal]**

**(Patent: PCT/SG2013/000027)**

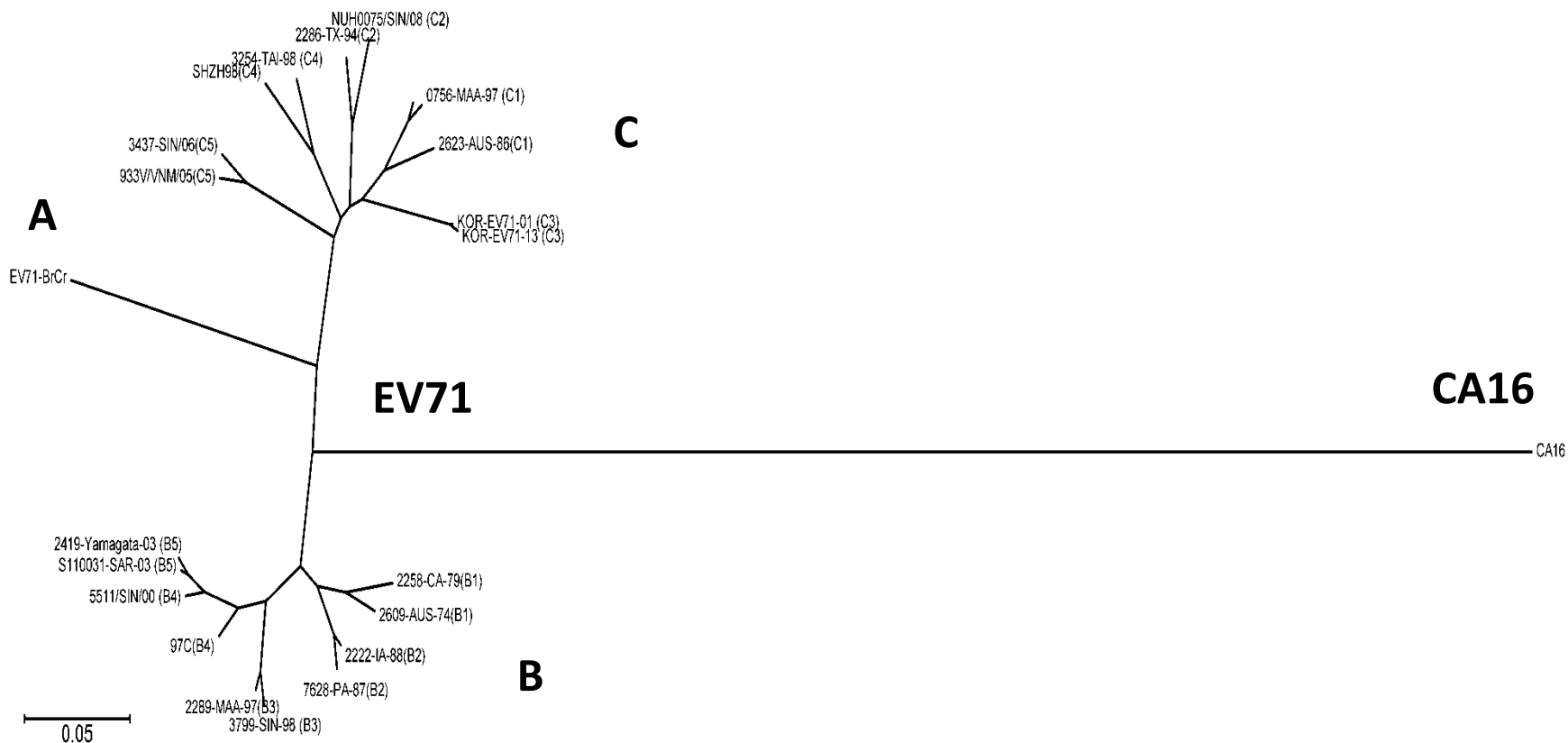


# Safety and Immunogenicity: Monkey's Study

**1 monkey: normal control**

**3 monkeys (2889M, 0791M and 2891F):  $10^7$  CCID<sub>50</sub> of EV71: TLLβP20**

**3 monkeys (2890M, 2202F and 2247M):  $10^8$  CCID<sub>50</sub> of EV71: TLLβP20**



**Need to Extend Protection against: other Genotypes of EV71 and CA16**

# Chimeric Enteroviruses

**EV71:TLLeC5 (PCT/SG2015/050106)**

**TLLeCA16 (PCT/SG2015/050106)**

# Final Candidate Vaccine

**An Oral Combined Live Attenuated Vaccine  
(EV71:TLLβP20, EV71:TLLeC5, and TLLeCA16)  
against Hand Foot and Mouth Disease  
due to EV71 and CA16**

# Future Prospect

## Formalin-Inactivated EV71 Vaccine

Implementation of a 2-dose regimen in China with ? a third booster dose.

### Issues Need to be Considered:

- **Multinational Efficacy Trials.**
- **Harmonization and Standardization of Antigen (Virus Strain).**
- **Co-ordinated Quality Control Reagents and Immunoassays.**
- **Co-administration of EV71 vaccine with the commercial paediatric pentavalent vaccine.**
- **A global surveillance: Circulating genotypes/subgenotypes or new serotype emerging from EV71 and CA16.**
- **HFMD due to CA16 virus.**

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# Acknowledgement

**Virologists and Molecular Biologists**

# Thank You