

Seroepidemiology of enterovirus 71 and Coxsackievirus A16 infections in population of various age-groups, Nakhon Ratchasima province, Thailand



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Introduction:

Hand foot and mouth disease (HFMD) is a common infectious disease in childhood in Asia-Pacific region. The disease is caused by many enteroviruses, in particular, enterovirus 71 (EV71) and coxsackievirus A 16 (CA16). However, EV71 has been implicated in cases with severe complications and deaths. In Thailand, HFMD has become a notified disease by the Ministry of Public Health since 2001. HFMD outbreaks occur in many provinces every year, but death is not common. There were 3,961 cases with 7 deaths in 2006; and in 2013, there were 4 deaths among 45,853 cases

Objective:

This seroepidemiological study was conducted in 2013 and aimed to determine the prevalence of antibodies to enterovirus 71 (EV71) and Coxsackievirus A16 (CA16) in people of various age-groups by cytopathic effect (CPE) based microneutralization assay.

Materials and method:

Sera: A total of 1,327 serum samples comprised 494 leftover samples from the hospitalized pediatric patients aged < 5 yrs; and 833 samples from healthy individuals of age older than 5 who lived in the community.

Microneutralization assay: The plate plan is shown in Figure 1.

Sera were 2-folded serially diluted to obtain the dilutions of 1:10 to 1:1280

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The test viruses : 100 TCID₅₀/reaction
SiICRC10/TH/2011 (EV71-B5/2011) and
SiICRC03/TH/2012 (CA16-B1a/2012)

incubated at 37 C for 2 hr

Inoculate the serum-virus mixtures
onto Vero cell monolayers in duplicate

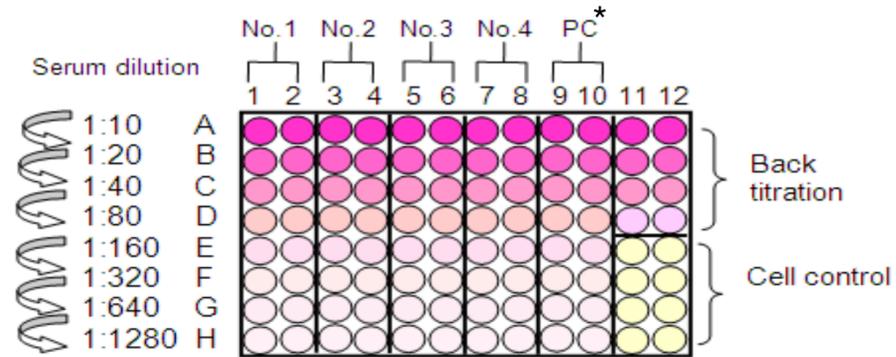
Incubated at 37 C, 5 % CO₂

Observe for CPE after 5-6 days of incubation (Figure 2).

Antibody titer is defined as the reciprocal of highest serum dilution that yields <50% CPE. Titers of ≥ 10 is considered positive.

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PC* = Positive control

Figure 1. Plate plan for microneutralization assay

Result:

Approximately 90% of people in all age-groups studied had antibody to EV71. Highest geometric mean titers (GMT) of 266.3 for EV71 antibody were noted in children of age younger than 5; and waning of antibody level was clearly seen with increasing age, i.e., GMT of 141.9 and 121.5 in the age-groups of 5 to <18 and ≥ 18 , respectively. The age-group < 5 yrs was verified in more detail; and it was shown that the lowest positive rate of 83.6% and the highest positive rate of 97.4% were found in the age-group 6-11 mos. And 49-59 mos, respectively (Figure 3).

In contrast, the number of people with CA16 antibody was very low. Of 983 samples studied, the infection rate of 2.4% (12 of 494) was found in the age-group <5 years, 11.8% (34 of 289) in the age-group between 5 and <18 years, and none in 200 subjects of the age-group ≥ 18 (Table 1).



Figure 2. Characteristics of CPE at 4+ degree

Table 1. Seropositive number. and GMT value of antibody against EV71- B5/2011 or CA16-B1a/2012 in different age groups (N = 1,327)

Groups	Age	EV71-B5/2011			CA16-B1a/2012		
		N	No. pos (%)	GMT	N	No. pos (%)	GMT
Hospital	0-<5	494	444 (89.9)	266.3	494	12 (2.4)	5.1
	5-<18	615	589 (95.8)	141.9	289	34 (11.8)	6.0
Community	≥ 18	218	209 (95.9)	121.5	200	0 (0)	5

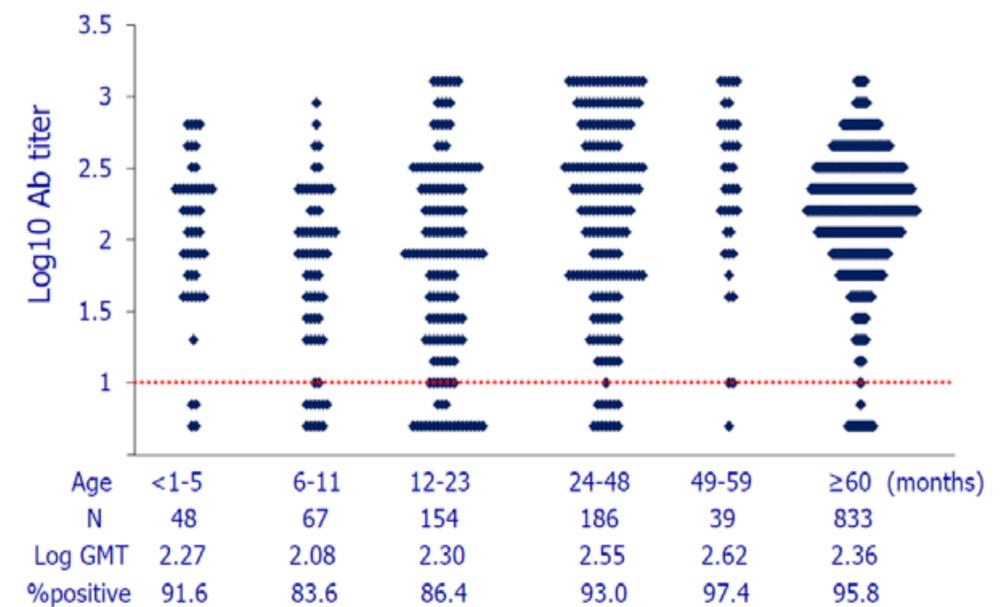


Figure 3. Neutralizing antibody levels against EV71 in each age group

Conclusion:

Infection rate of EV71 and CA16 in the population was determined from the prevalence of positive neutralizing antibody. This study demonstrated that EV71 infection rate is much higher than CA16 in all age-groups studied. The lower positive rate of EV71 antibody in the age-group 6-11 mos. is concordant to the data from MOPH in 2012 such that HFMD occurred in very young children, i.e, 43% of the patients were 1 yr old, and 28% were 2 yrs old.