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CHARACTERISTIC OF ENTEROVIRUSES CIRCULATING IN SOUTH VIETNAM AND THEIR ROLE IN ETIOLOGY OF INFECTIOUS DISEASES

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DISSERTATION

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SECTION 1. INTRODUCTION

Human enterovirus infection is united infectious diseases with different clinical forms caused by viruses of the Enterovirus A-D species, which belong to *Enterovirus* genus of the *Picornaviridae* family [148]. Human enteroviruses (HEV) are devided into four types of A-D (Enterovirus alphacoxsackie, betacoxsackie, coxsackiepol, deconjuncti) and more than 100 types. Three species C types of enteroviruses called polioviruses are the etiological agents of poliomyelitis – neurological infection with typical clinic of acute flaccid paralysis which can result in residual paralysis. Enterovirus A71, enterovirus D68, some Echoviruses and Coxsackieviruses also can result into neurological complications (meningitis and encephalitis).

In 1988, the World Health Assembly adopted a resolution to eradicate poliomyelitis in the world by the year 2000. The Plan of Action for the Global Elimination of Poliomyelitis was approved in 1989. According to the recommendations of the World Health Organization (WHO) the most effective way to found paralytic poliomyelitis is the surveillance of Acute Flaccid Paralysis syndrome and obligatory laboratory diagnostics of revealed cases. To date five WHO regions from six are certified as polio free regions [103]. Wild type 1 poliovirus now circulates only in Afghanistan and Pakistan.

Importance of the research topic

The number of types of non-polio enteroviruses (NPEV) is large, more than 100 types are currently known. Enteroviruses (EV), which are the causative agents of enterovirus infection (EVI), are characterized by high variability, which is associated with two mechanisms of viral evolution. Mutations play an important role in the formation of new enterovirus variants, a high frequency of nucleotide substitutions in different parts of the virus genome can lead to changes in a number of properties. The process of recombination, when two viruses exchange parts of genetic material [139] and hybrid forms of viruses are formed, which contain features of two parent strains, which also changes the properties of the virus.

Laboratory confirmation of the diagnosis of enterovirus infection is carried out using virological and molecular biological methods. Isolation and identification of

viruses is carried out by the classical virological method on certified cell lines. The type of enterovirus is determined in a neutralization reaction using specific diagnostic sera. This is a labor-intensive and lengthy process due to the large number of types of enteroviruses and depends on the availability of appropriate sera. Therefore, molecular biological methods are often used for the detection and subsequent identification of these viruses: polymerase chain reaction (PCR) and sequencing of the virus genome [93]. Genome sequencing allows us to determine both the type and genotype of the enterovirus, which serves to establish the etiology of outbreaks and group diseases of enterovirus infection. Sequencing of the virus genome also allows us to clarify the phylogenetic relationships and geographic origin of various strains of enteroviruses. The search for and identification of enteroviruses in different categories of examined individuals, as well as the study of the molecular genetic characteristics of enteroviruses circulating among people at different periods of time in individual provinces of South Vietnam, is an urgent scientific task.

Enterovirus infection (EVI) can vary in clinical manifestations and severity: from asymptomatic infection or mild febrile illness to serious multisystem diseases, accompanied by damage to the cardiovascular and central nervous system. Clinical forms with paralysis, meningitis or meningoencephalitis because of their severity can end in death. Since enteroviruses of the same type can cause diseases with different clinical forms, diagnosis can be difficult.

Due to the peculiarities of the molecular organization of enteroviruses, strains with increased neurovirulence, atypical pathogenicity and ability to transmit can be formed in the process of their evolution. Enterovirus infection, accompanied by neurological complications, poses a great danger to human health and is one of the most pressing health problems in South Vietnam and the country as a whole.

The degree of the research topic development

The establishment of a national polio program in Viet Nam has been critical to the country’s elimination plan. Viet Nam was accredited as polio-free along with other countries in the WHO Western Pacific Region in 2000. The final phase of polio eradication was achieved through the widespread use of oral poliovirus vaccine in

Viet Nam for routine three-dose immunization of children aged two, three and four months. Since 2019, the vaccination schedule has been against poliomyelitis changed, an additional vaccination was added using an inactivated poliovirus vaccine for routine immunization of children aged five months. The result of the measures taken was a decrease in the circulation of vaccine polioviruses, which can be a source of the formation of vaccine-derived polioviruses (VDPV), approaching wild strains in virulence. The number of cases of acute flaccid paralysis associated with infection with polioviruses (vaccine or VDPV) also decreased. The ecological “niche” that was formed as a result of the measures taken began to be filled with non-polio enteroviruses (NPEV), which previously and currently widely circulate among the population of the country. This caused an increase in the number of cases of enterovirus infection, complicated by the development of acute flaccid paralysis.

Until 1997, the leading cause of viral diseases of the central nervous system in Vietnam was Japanese encephalitis virus. Since the inclusion of the Japanese encephalitis vaccine in the Expanded Program on Immunization in Asia, there has been a significant decrease in the proportion of Japanese encephalitis in the structure of neurological infections. Now, the leading etiological agents of severe neurological diseases of a viral nature in Vietnam include dengue virus and enteroviruses [129] .

The high socio-economic significance of enterovirus infection, mainly enterovirus exanthema of the oral cavity and extremities (foot-and-mouth disease syndrome), is the cause of a significant economic burden in Vietnam [91]. The situation with mass outbreaks of this disease requires studying the patterns of development of the epidemic process of enterovirus infection, improving epidemiological surveillance, virological diagnostics of EVI and measures to prevent this infection

Surveillance of EVI in Vietnam is based on monitoring the incidence of enteroviral exanthema of the oral cavity and extremities - Hand, Foot and Mouth Disease (HFMD). The first cases of this disease, complicated by encephalitis, were reported in Vietnam in 2003 in Ho Chi Minh City during an outbreak of acute

encephalitis [136]. A sharp increase in HFMD incidence in Vietnam occurred in 2011, when 113,121 cases of the disease were registered, of which 170 were fatal [88]. HFMD-related morbidity and mortality in 2011 recorded in all parts of Vietnam, however, the largest proportion of illness and death were in South Vietnam; In 2012, the epidemic spread to the northern part. In 2013-2017 the number of patients and deaths from HFMD in Vietnam has decreased significantly, but the incidence rate has remained much higher than before the epidemic. In 2018 an increase in morbidity and mortality was again observed [46].

In 2011 The Ministry of Health of Vietnam classified HFMD as a serious infectious disease with high epidemic potential and included it in the list of infectious diseases subject to weekly reporting. In 2012 in South Vietnam, a surveillance system for enterovirus hand foot and mouth disease was created. It is based on a mandatory laboratory examination of severe and fatal cases of EVI and the study of isolated enterovirus strains [32]. In connection with the foregoing, the systematic monitoring of enterovirus circulation, the analysis of the spectrum of types and genotypes of enteroviruses isolated from EVI patients, as well as the analysis of the change in the dominant types of enterovirus infection pathogens, has important epidemiological and social significance. This is necessary for predicting the development of the EVI epidemic situation in South Vietnam, for the timely implementation of preventive measures, and for the development of vaccine prevention for the most widespread and severe clinical forms of enterovirus infection.

Purpose of the study To study the virological and molecular properties of enteroviruses, the characteristics of their circulation among the population of South Vietnam, and the role of enteroviruses in the etiology of infectious diseases.

Research objectives:

1. To determine the frequency of poliovirus and non-polio enterovirus isolation from patients with acute flaccid paralysis syndrome in South Vietnam.

2. To reveal the species and types of non-polio enteroviruses associated with non-polio enterovirus infection in South Vietnam, and to evaluate the role of

dominated in circulation non-polio enteroviruses as etiological agents of various clinical forms of enterovirus infection.

3. To evaluate the prevalence of various types of non-polio enteroviruses among the population of South Vietnam. To reveal the leading clinical form of non-polio enterovirus infection in South Vietnam and to determine the types of non-polio enteroviruses associated with this form of infection.

4. To determine the leading in circulation type of non-polio enterovirus and to evaluate the role of this enterovirus as etiological agents of various clinical forms of infection in South Vietnam.

5. To characterize the molecular features and phylogenetic relationships of enteroviruses most widely circulated in South Vietnam and frequently detected in patients with non-polio enterovirus infection.

Scientific novelty

Based on the analysis of the virological and molecular properties of enteroviruses isolated from patients with acute flaccid paralysis and enterovirus infection, the changes in types and genotypes of enteroviruses circulated among the population of South Vietnam over a long-term observation period were shown. The types and genotypes of enteroviruses that dominated in circulation and caused the outbreaks of enterovirus infection in different years were identified. For the first time, the analysis of the outbreaks and epidemics of enterovirus infection during the long-time period was done taking into account the different of clinical forms of this infection in some provinces of South Vietnam. Using clinical, epidemiological and virological analysis, an assessment was made of the role of the leading types and genotypes of enteroviruses as etiologic agents of various clinical forms of enterovirus infection in South Vietnam.

Theoretical and practical significance of the obtained results

An integrated approach to enterovirus infection diagnostics using virological and molecular research methods has provided new systematized data on the circulation of non-polio enteroviruses of different types and genotypes in South Vietnam over a long observation period. Research aimed at studying the properties of enteroviruses is a prerequisite for preventing risks associated with enterovirus infection in South Vietnam. It is necessary to establish systematic monitoring of enterovirus circulation, paying special attention to the spread of new variants of enteroviruses of different types. This will allow predicting the development of increases in enterovirus infection and promptly taking preventive measures based on new technological solutions, including in the field of vaccination against this infection.

Research methodology

The methodological basis of the study was a combination of virology and molecular biological research methods. Enteroviruses were isolated using standardized cell lines (RD, L20B and Vero). Poliovirus identification was performed by rRT-PCR ITD test. Molecular studies included RNA extraction, reverse transcription PCR, genome sequencing, and phylogenetic analysis of enteroviruses.

Statements submitted for defense

1. The isolation of polioviruses, mainly vaccine-like except two VDPV2, from patients with acute flaccid paralysis did not exceed 1%. The isolation from such patients 11,6% of non-polio enteroviruses was connected with their widespread circulation in South Vietnam and with neurologic complications of non-polio enterovirus infection.

2. The predominance in circulation of species A enteroviruses (Enterovirus alphacoxsackie), including EVA71, determined the domination of HFMD among clinical forms of non-polio enterovirus infection. The changes in types and genotypes of species A enteroviruses in the course of long-term circulation led to periodic peaks of enterovirus infection in South Vietnam.

3. Enterovirus 71 was the main etiological agent of the leading form of non-polio enterovirus infection in South Vietnam. Enterovirus 71 was found as well as enteroviruses of species B (Enterovirus betacoxsackie) in patients with enterovirus meningitis and acute flaccid paralysis syndrome.

4. Most studied enterovirus 71 strains circulated in South Vietnam belonged to the C4 genotype, some strains were represented by B5 genotype. Identified strains of Coxsackievirus A10 isolated from patients mainly belonged to F3 genotype, one strain belonged to F1 genotype.

Personal contribution of the author

The author's personal contribution consists of planning and direct implementation of all virological studies. Biological material obtained from patients with AFP and EVI syndrome, as well as molecular genetic studies and analysis of the results obtained in order to establish patterns of development of the epidemic process in enterovirus infection. The author carefully analyzed the scientific literature and own data. Statistical processing of the data obtained, generalization of research results, preparation of materials. The author also contributed to the deposition of nucleotide sequences in the international GenBank database and to publications.

The level of reliability and implementation of the results

The reliability of the results of the work is ensured by the use of modern research methods, a significant volume of research conducted, a large array of processed data and the use of statistical methods for processing the obtained data.

The results of the work were tested throughout the entire period of the study. The main provisions of the dissertation were presented at 4 conferences.

The results of this work were used in analytical reviews:

1. The presentation “Enterovirus Infection in South Vietnam.” in the IV International conference on combating new coronavirus infection and other infectious diseases and International Conference “Results and Future Potential for Cooperation Between Joint Research Centers in Africa, Asia and South America for the Study and Prevention of Infectious Disease” in December 2023, St. Petersburg

2. The presentation “Enterovirus 71 infection of hand foot and mouth disease, in South Vietnam” the International Symposium "100 years carrying the name Louis Pasteur" in June 2023, Saint Petersburg.

3. The presentation “Serotyping of Hand, Foot, and Mouth Disease (HFMD)- Associated Enteroviruses circulating in Southern Vietnam, 2016–2018” the International Symposium " Russian-Vietnamese conference on November, 2019, Moscow

4. The presentation “Circulation of Coxsackievirus A in hand-foot-mouth disease in Southern Vietnam, 2015-2016” International Conference on Molecular bases of Epidemiology, Diagnostics, Prevention and Treatment of infectious diseases. Dedicated to the 110th anniversary of St. Petersburg Pasteur Institute December, 2018, Saint Petersburg

Publications

The main statements of the dissertation are reflected in 10 scientific works, in the Web of Science and/or Scopus databases.

1. Nguyen N.T.T., Donato C., Trang V.T.H., Kien N.T., Trang P.M.M.T., Khanh T.Q., Nguyet D.T., Sessions O.M., Cuong H.Q., Lan P.T., Huong V.T.Q., van Doorn H.R., Vijaykrishna D. Evolution and Spatiotemporal Dynamics of Enterovirus A71 Subgenogroups in Vietnam. J Infect Dis, 2017, vol. 216, no.11, pp. 1371-1379.

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Contacts of Index Cases in Dong Thap Province, Southern Vietnam. Biomed Res Int, 2020, no.1, 9850351.

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8. Romanenkova N.I., Golitsyna L.N., Nguyen T.T.T, Ponomareva N.V., Leonov A.V., Kanaeva O.I., Zverev V.V., Selivanova S.G., Rozaeva N.R., Luong M.T., Bichurina M.A., Novikova N.A. Epidemiological and etiological aspects of enterovirus infection in Russia and Vietnam// Russian Journal of Infection and Immunity. – 2021. – V. 11., N. 5. – P. 905-916.

9. Nguyen T.T., Chiu C.H., Lin C.Y., Chiu N.C., Chen P.Y., Le T.T.V., Le D.N., Duong A.H., Nguyen V.L., Huynh T.N., Truong H.K., Phan T.L., Nguyen T.T.T., Shih S.R., Huang C.G., Weng Y.J., Hsieh E.F., Chang S., Chen C., Tai I.C., Huang L.M. Efficacy, safety, and immunogenicity of an inactivated, adjuvanted enterovirus 71 vaccine in infants and children: a multiregional, double blind, randomized, placebo-controlled, phase 3 trial. Lancet, 2022, vol. 399, no.10336, pp. 1708-1717.

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Implementation of work results

The results of this work are presented in two textbooks for specialists such as pediatricians, infectious disease specialists, virologists and epidemiologists.

1. Reference manual “Surveillance and control of viral infections relevant to the Socialist Republic of Vietnam”. St. Petersburg. 2018. 72 pages.

2. Reference manual "Improving Epidemiological Surveillance and Diagnostics of Enterovirus Infection in the Socialist Republic of Vietnam." St. Petersburg, 2022, 80 pages (Russian, Vietnamese). ISBN978-5-904405-56-4.

Nucleotide sequences have been deposited in the international GenBank database. Complete nucleotide sequences of the VP1 genome region of 67 EV-A71 viruses are submitted to the International GenBank under accession numbers MW139687–MW139744, OR947996–OR948003.

The results obtained in this study are useful to many specialists (epidemiologists, virologists, infectious disease specialists, and others). They are used in lectures at medical universities in Vietnam and can be used in other medical schools.

Volume and structure of the dissertation

The dissertation is presented on 120 typewritten pages (132 in Russian), includes 22 tables and 29 figures. The work consists of an introduction, literature review, description of materials and research methods, four chapters of original research, discussion of the obtained results, conclusion, conclusions and a list of cited literature. The list of references includes 152 sources.

SECTION 2. MAIN PART

THE LITERATURE REVIEW

Enterovirus infection affects millions of people worldwide every year. Its pathogens are members of the *Picornaviridae family*, genus Enterovirus. Enteroviruses can be associated with a variety of acute illnesses, from mild fever to severe forms of infection. Sometimes an enterovirus infection can be protracted due to the long-term persistence of enteroviruses in the patient's body. Among the representatives of the genus of enteroviruses, polioviruses are the most historically known and important because they are the cause of poliomyelitis, one of the most dreaded diseases, clinically manifested as acute flaccid paralysis. Hundreds of thousands of children suffered from poliomyelitis every year before the development of poliovirus vaccines. Enteroviruses undergo extensive genetic changes through recombination and reassortment. The genus Enterovirus is divided into 15 species based on phylogenetic analysis and includes 7 species that affect humans, of which 3 species are representatives of the genus Rhinovirus [116].The genus Parechovirus has two species that are pathogenic for humans: Parechoviruses A and B. Previously, these Parechovirus were classified as enteroviruses (ECHO 22, 23), but during a detailed study of the genome, significant differences were revealed in the genomic regions responsible for replication and translation, so they were isolated into a separate genus [36]. There are currently more than 250 types of enteroviruses, including a number of types of rhinoviruses and three types of polioviruses, and 16 types of parechovirus, of which 6 are pathogenic to humans [27, 95]. Diversity within these virus families is also reflected in a wide range of disease symptoms, including asymptomatic infections (most common), non-specific rash and fever, herpes and hand-foot-mouth disease, and respiratory conditions such as pneumonia [152]. While detecting viral infections in the gastrointestinal, blood, and lower respiratory systems provides clear evidence of current infection, detecting them from the throat and upper respiratory tract only indicates past infection, as asymptomatic infections can persist for weeks to months.

1.1. General characteristics of enteroviruses, classification, genome structure, replication and genetic transformation of enteroviruses

Enteroviruses are common viruses that cause many diseases annually, for example, 10-15 million cases in the United States [124]. Enteroviruses affect the oropharynx and gastrointestinal tract, they cause a variety of diseases. Most enteroviruses are associated with mild or asymptomatic cases. According to many studies, enterovirus infection can be accompanied by hand, foot and mouth disease manifestations, in most cases with mild clinical symptoms. Some enteroviruses are able to affect the central nervous system and cause serious neurological diseases in patients, such as meningitis, encephalitis or paralysis [77]

The seasonality of enterovirus infection has been noted for a long time: most enterovirus infections in temperate climates occur in summer and autumn. However, in tropical and subtropical climates, the incidence of EVI continues all year round [47, 99]. Enteroviruses (except rhinoviruses) often give a high incidence in the summer. This incidence also varies depending on the types of enteroviruses [49]. Many types of EV circulate with different frequencies in individual years, and the prevalence of a particular type can fluctuate. Outbreaks usually occur periodically, every few years. Children are most susceptible to these viruses.

Enteroviruses are usually detected in respiratory secretions (saliva, sputum or nasal mucus) and in the feces of infected individuals, as well as in cerebrospinal fluid and blood. The type of material examined depends on the clinical manifestations of the disease [37].

Enteroviruses are spherical, non-enveloped viruses with a diameter ranging from 28 to 30 nm. They have a single-stranded positive-sense RNA genome [83]. The genomes of enteroviruses are approximately 7.5 to 8.0 kb in length and contain an open reading frame (ORF) surrounded by highly structured non-translated regions (UTRs) at the 5' and 3' ends. The 5' UTR includes a cloverleaf-like RNA structure followed by an internal ribosome entry site (IRES) [76]. The IRES is a highly structured RNA element that directly interacts with ribosomes, allowing the viral protein translation to occur independently of the cap-dependent mechanism used by

host cells. The ORF encodes a polyprotein that is processed into individual viral proteins by viral proteases [31].

Enterovirus infections are often associated with a specific type of virus. The most common clinical forms specific serum type are:

- Poliomyelitis: polioviruses types 1-3 (PV 1-3)
- Acute flaccid paralysis and other poliomyelitis-like diseases: EVD68, EVA71, ECHO 11, etc.
- Myocarditis: CVA, CVB
- Hemorrhagic conjunctivitis: EVD 70
- Pneumonia: EVD 68, rhinoviruses
- Herpangina: CV-A, EV-A71
- Hand, foot and mouth disease: CVA 16, EVA 71
- Upper respiratory tract infections: EVD 68, rhinoviruses
- Exacerbation of chronic respiratory diseases (chronic obstructive pulmonary disease, cystic fibrosis, asthma): rhinoviruses
- Aseptic meningitis: CVA 9, CVB, ECHO viruses of various types, EV 71 [63, 82, 92, 104, 116, 127].

Enteroviruses belong to the *Picornaviridae* family, which includes two genera: *Enterovirus* and *Rhinovirus*. They are small, contain single- stranded RNA, have a cuboidal symmetrical capsid, and do not have an envelope. The genus *Enterovirus* includes 4 species A , B, C, and D [65]:

Poliovirus: Poliovirus affects the epithelial cells of the oropharynx and intestines, so it can be isolated from these organs. Among the polioviruses, there are three types: 1, 2, 3 [107]. All of them cause poliomyelitis - an acute disease that affects the central nervous system. The virus can destroy the motor neurons of the spinal cord (anterior horns) and the cerebral cortex, causing flaccid paralysis. During an epidemic, 90-93% of infections are latent, 4-8% have a mild course with respiratory and intestinal symptoms, and only 1-2% of infected individuals develop a paralytic syndrome.

Coxsackieviruses include 29 types [13]. They differ from other enteroviruses in their ability to cause disease in mice; other enteroviruses are either nonpathogenic in laboratory animals or infection is not clinically evident. Coxsackieviruses are divided into two groups, group A and group B, with varying ability to cause paralysis in neonatal mice [53]. Coxsackie A viruses infect skeletal and cardiac muscle, while Coxsackie B viruses replicate in a wide range of cells and tissues, including the central nervous system, liver, pancreas, and other cells [53, 99]. Coxsackie B viruses reproduced more actively in mice with selenium insufficiency [5].

Echoviruses (enteric cytopathogenic human orphan viruses) include 33 types. They are also found in the gastrointestinal tract, causing cytopathic effects in cell cultures, but do not cause pathological lesions in mice [53, 99, 112]. Echoviruses are associated with a variety of pathological manifestations from mild respiratory disease to brain damage [44].

Among the enterovirus genera, the Rhinovirus species is the species capable of infecting both the upper and lower respiratory tract [121]. Studies have found that this virus at a temperature of 36 degrees Celsius is an ideal environment for effective growth. However, rhinoviruses (and other respiratory enteroviruses) are most effective at temperatures of 33 degrees Celsius, which may contribute to they become upper respiratory pathogens. Although higher temperatures have not been shown to affect viral replication, they do affect host cell responses, creating a less favorable environment for the virus [121].

In addition, non-rhinovirus enteroviruses are considered to be the most common viral pathogens that affect the central nervous system [121]. The blood-brain barrier protects the CNS from most infections, but enteroviruses are able to cross it [138]. The route of infection begins through the gastrointestinal tract. These viruses have a unique characteristic that distinguishes them from other rhinoviruses and picornaviruses: the ability to survive in environments with a pH of less than 3. This helps them overcome the acidic environment of the stomach and reach other organs where they can initiate disease [102]. After the development of molecular methods of typing and revision of the old limited classification schemes enteroviruses are divided

into serotypes depending on the organization of their genome, similar in nucleotide sequences and biological properties.

Following the development of molecular typing methods and revision of old limited classification schemes, enteroviruses are divided into types based on their genome organization, nucleotide sequence similarity, and biological properties. The current classification of enteroviruses is constantly being revised and refined. According to the latest changes in the International Committee on Taxonomy of Viruses (ICTV) database, the picornavirus family includes 12 enterovirus species [72]: 4 species (former A, B, C, D, now *Enterovirus alphacoxsackie, Enterovirus betacoxsackie, Enterovirus coxsackiepol, Enterovirus deconjuncti*) of human enteroviruses (HEV), including polioviruses of three types belonging to species *Enterovirus coxsackiepol*, 8 species animal enteroviruses of various species (E, F, G, H, I, J, K, L) and since 2008 3 species rhinoviruses (former A, B, C, now *Enterovirus alpharhino, Enterovirus betarhino, Enterovirus cerhino*) [57,149]. The types differ in the host immune response, the receptors used, and, to a lesser extent, the spectrum of clinical manifestations that occur after infection. Currently, more than 120 types of human enteroviruses are distinguished within the four types [59].

Table 1. Classification of human enteroviruses [21]:

Enterovirus alphacoxsackie (HEV-A)	Coxsackievirus A 2-8, 10, 12, 14, 16
	Enterovirus 71, 76, 89-92, 114,119-125
Enterovirus betacoxsackie (HEV-B)	Coxsackievirus A 9
	Coxsackievirus B 1-6
	Echovirus 1-7, 9, 11-21, 24-27, 29-33
	Enterovirus 69, 73-75, 77-88, 93, 97, 98, 100, 101, 106, 107, 110-114.
Enterovirus coxsackiepol (HEV-C)	Coxsackievirus A 1, 11, 13, 15, 17-22, 24
	Enterovirus 95, 96, 99, 102, 104, 105, 109,113, 116-118
	Poliovirus 1-3
Enterovirus deconjuncti (HEV-D)	Enterovirus 68, 70, 94,111,120

1.2 Replication and genetic transformation of enteroviruses

Morphological changes in the cell monolayer during poliovirus infection were characterized in 1958 using an electron microscope, and three stages were identified [64]. In 1987, also using electron microscopy, the stages of viral RNA replication in the cell and the process of vesicle formation upon contact with membrane structures were demonstrated [8].

Enterovirus replication within the cell depends on specialized membrane structures called replication organelles (ROs) that are formed during infection, but their origin remains unclear. A hallmark of the replication of many RNA viruses is the formation of ROs during infection [134]. However, each viral order has different mechanisms for generating specific morphological structures. Typical examples include the formation of membrane vesicles, commonly found in dengue virus [140] and Zika viruses [20], Other viruses, such as hepatitis C virus [115] or severe acute respiratory syndrome viruses such as coronaviruses, form double-membrane vesicles in a number of other structures, and can be found in the combines the outer membrane with the endoplasmic reticulum or may appear alone.

The replication organelles (ROs) of Enterovirus not only represent a unique structure but also have distinct structures and functions within the infected cells. The proliferation and development of cells during membrane remodeling rely on lipids such as cholesterol and phosphatidylcholine, which are incorporated into ROs through the cellular lipid metabolism and regulated by regulated intracellular trafficking, lipid droplet (LD) metabolism, and lipid synthesis [54, 134, 137]. During subsequent transmission, enteroviruses generate ROs in the form of single-membrane tubes (SMTs), which further develop into double-membrane vesicles (DMVs) and multilayered structures during infection [6, 74, 134] . While enterovirus ROs exist within tight membrane clusters, both SMTs and DMVs have distinct and separate structures, not forming a continuous membrane network. In another genus of *Picornaviridae*, similar membrane morphology was found in cells infected with cardioviruses [81].

Proteins 2B, 2C and 3A have hydrophobic regions and participate in the formation of vesicles. It is possible to create a double membrane structure of vesicles with biochemical properties similar to that obtained during enterovirus infection only with parallel expression of both 2BC and 3A proteins with the participation of Golgi complex membranes [125]

Some studies have found some structural mechanisms of replication due to modification of the membranes of their secretory pathways. One possible explanation for this intense inhibition of retrograde transport from the endoplasmic reticulum membrane to the Golgi complex by enterovirus infection is the already mentioned membrane redirection [23, 141]. In addition, treatment with brefeldin A has also been studied to be extremely sensitive to enterovirus replication. Brefeldin A is a secretory inhibitor commonly used in clinical practice [55, 80].

1.3. General characteristics of polioviruses

Throughout the history of enterovirus studies, poliomyelitis has been the most severe disease caused by them. Poliomyelitis is caused by polioviruses (Poliovirus) [109], of which there are 3 types. All three can cause the disease:

- Type 1: the prototype Mahoney strain , isolated in 1941 [118], or the Brunhilde strain [111], is the leading cause of paralytic diseases, accounting for 90% of all cases of paralysis.
- Type 2: prototype strain Lansing, isolated in 1943 [120].
- Type 3: prototype strain Leon, isolated in 1937[118] .

Poliovirus consists of an RNA genome and a protein capsid. The genome is a single-stranded positively charged RNA (+ssRNA) approximately 7500 nucleotides long [43, 109]. The viral particle has a diameter of about 30 nm with icosahedral symmetry. Due to the short genome, the virus has only RNA and a protein coat without a lipid membrane. Many researchers consider the poliovirus to be the simplest virus. In terms of structure, poliovirus, when viewed under an electron microscope, has a spherical shape with a diameter of 27 nm, a molecular weight of

6.8x10 ^{6} daltons, does not have a super capsid, and consists of a capsid protein with a stable structure that covers the viral RNA [94].

The structure of the poliovirus genome [7, 21, 100]:

3Dpol, an RNA-dependent RNA polymerase whose function is to create multiple copies of the viral genome

2A ^{pro} and 3C/3CD ^{pro}, proteases that cleave the viral polypeptide

VPg (3B), a small protein that initiates the synthesis of the positive and negative strands of viral RNA

2BC, 2B, 2C (ATPase), 3AB, 3A, 3B proteins that make up the protein complex necessary for viral replication.

VP0, which is further cleaved into VP2 and VP4, VP1 and VP3 - proteins of the viral capsid (Figure 1).

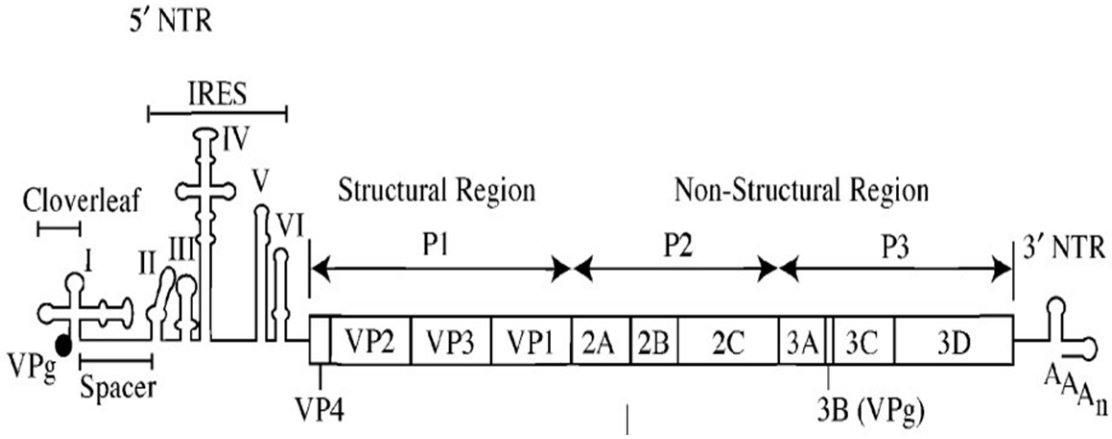

Fig. 1. The structure of the poliovirus type 1 genome (Mahoney) [21].

The mechanism of poliovirus infection involves binding to a specific immune globulin-like receptor called CD155 (commonly referred to as the poliovirus receptor or PVR) on the surface of human cells [21, 58]. The interaction between poliovirus and CD155 facilitates conformational changes in the virus particle that are necessary for viral entry [58]. Following attachment to the host cell membrane, entry of the viral nucleic acid is thought to occur in one of two ways: by the creation of pores in the cell membrane through which the RNA is injected into the host cell cytoplasm, or by virus uptake via receptor-mediated endocytosis [21].

Translation, transcription, and replication of the genome, which are a single process, result in the synthesis of (+)RNA. Replication of infectious (+)RNA requires the transcription of multiple copies of (−)RNA, which are then used as templates for the synthesis of (+)RNA. Replication intermediates, which are an association of RNA molecules consisting of messenger RNA and several elongating RNAs of varying lengths, are created in both (−)RNA and (+)RNA replication complexes (Figure 2).

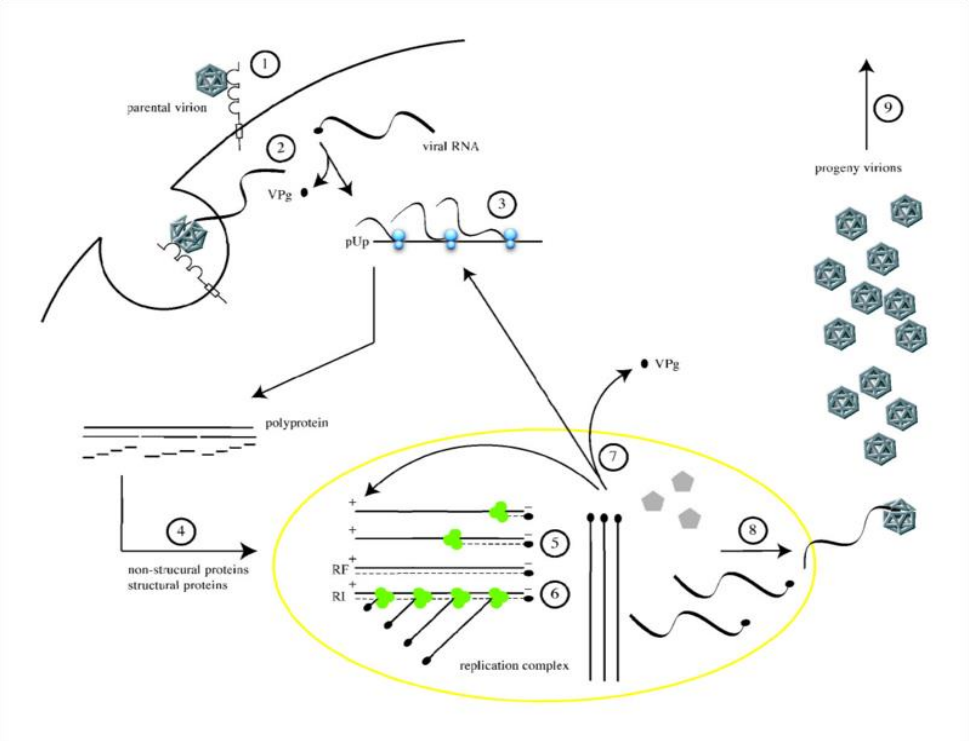

Figure 2. Cellular life cycle of poliovirus.

Initiated by binding of a poliomyelitis to the cell surface macromolecule CD155, which functions as a receptor (1). Uncoating of viral RNA is mediated by receptor-dependent destabilization of the viral capsid (2). Cleavage of the viral VPg protein is carried out by cellular phosphodiesterase, and viral RNA translation occurs by a cap-independent (IRES-mediated) mechanism (3). Proteolysis of viral polyproteins produces mature structural and nonstructural proteins (4). To generate a double-stranded replicative form (RF) of RNA, the positive-sense RNA serves as a template for the synthesis of the complementary negative-sense strand (5). The initiation process of many positive strands from a single negative strand produces a partially copied intermediate (6). Meanwhile, RNA molecules of positive sense significance are synthesized to serve as templates for translation (7), or they can associate with

precursor capsid proteins to undergo packaging and generate the cleavage of VP0, leading to the formation of mature virions (8). Eventually, the lysis of infected cells releases infectious progeny virions (9) [21].

Fully assembled poliovirus leaves the host cell by lysis 4–6 h after the onset of infection in cultured mammalian cells. The mechanism of virus release from the cell is unclear, but each dying cell can release up to 10,000 virions. In addition, there is experimental evidence for the release of poliovirus particles without disruption of the cellular monolayer through the cytoskeletal microtubule system [130].

1.4. Clinical and epidemiological features of enterovirus infection. Pathogenesis of enteroviral infections

Enteroviruses often cause mild or asymptomatic disease. More than 90% of enterovirus infections will be either asymptomatic or have nonspecific symptoms such as sudden fever. However, if enteroviruses affect the central nervous system, they can cause severe disease [110]. Apart from poliomyelitis, aseptic meningitis and encephalitis are the most common complications involving the central nervous system and are most often caused by echoviruses, coxsackie B viruses and other B-type EVs [145]. Aseptic meningitis may also result from influenza, herpes, mumps or arbovirus infections in addition to enteroviruses, but enteroviruses are the most common cause [138]. Other types of EVs can also cause severe central nervous system disease, such as the EVA 71 virus [50] In addition, enteroviruses cause diseases such as herpangina, exanthema of the mouth and extremities, myocarditis, acute hemorrhagic conjunctivitis and acute respiratory viral infections [145]. Some clinical manifestations include ulcers on the legs, arms, mouth, fever and tremor [60]. Clinical manifestations and prevalence rates also vary depending on the type of enterovirus [147].

Typical sites of infection are usually the epithelial cells of the respiratory or gastrointestinal tract [85], followed by viral dissemination in the blood, leading to secondary foci of infection in tissues such as the spleen, liver, bone marrow, skin, and heart [77]. It is suggested that enteroviruses can bind to a variety of cell surface receptors, which accounts for the wide range of clinical manifestations of infection. A

number of receptors have now been discovered that EVs can use to enter the host cell [4]. Secondary infection in the central nervous system can lead to aseptic meningitis or, less commonly, encephalitis or paralysis [38]. Poliovirus reaches the muscles, from where it enters motor neurons via receptor-mediated endocytosis and reaches the neuronal cell body via retrograde axonal transport [77, 94]. Retrograde transport is inefficient, which may explain the small percentage of infected individuals who develop nervous system complications [70]. In addition, specific tissue infections may lead to pneumonia or myocarditis. Disseminated infection may present with a rash, nonspecific muscle pain, or severe multiorgan disease in infants. The virus usually enters the body through the mouth or nasopharynx [77]. The incubation period for most cases of EVD is 3 to 10 days [147]. Cases with mild or no symptoms are often the result of the host's immune system controlling the infection. Some enteroviruses can cause pneumonia and other respiratory diseases. This is especially true for EVD 68, which is biologically related to rhinoviruses and was considered rhinovirus 87 before the widespread introduction of molecular genetic typing methods [68, 82].

Enteroviruses are most commonly spread by contact with secretions such as saliva, sputum, or mucus from an infected person, vesicular fluid, or the feces of a patient [98]. Transmission occurs from one host to another. Although there is evidence of zoonotic transmission (both human-to-animal and animal-to-human), the virus is transmitted primarily from person to person via the feces-oral route [32]. The virus can be detected in the stool and pharynx of a patient several days before illness, with viral excretion peaking one week after illness onset [152], and the virus is then excreted in the stool for several weeks. Enteroviruses are typically highly transmissible, have high infectious potential, and have complex transmission routes. Within a short time, an enterovirus can cause a large outbreak among infected people.

In published studies of gastrointestinal tract specimens, the mean prevalence of EV was 6% among healthy children with no evidence of EV, 4.9% for cerebrospinal fluid specimens, and 3.4% for respiratory tract specimens. In studies of stool

specimens from patients with acute diarrhea, the prevalence of NPEV was approximately 17% [108]. Both types A and B were detected in both gastrointestinal and respiratory tract specimens.

Symptomatic enterovirus infection can potentially develop in humans of any age, but children under 10 years of age, especially during epidemics, demonstrate higher susceptibility [84]. Prevalence rates of EV recorded in studies involving adults over 18 years of age are significantly lower and range from 0% to 3.8% [14, 148]. While children under 6 months of age usually do not get sick, as they are protected by maternal immunity [63, 92, 104, 116, 127]. The likelihood of severe disease is higher in people with concomitant diseases, pregnant women, newborn or premature infants, as well as in people under stress due to hypothermia or malnutrition, and in immune compromised individuals [63].

1.4.1. Clinical features of enterovirus infection

Although millions of people are infected with enteroviruses every year, the majority of these individuals only experience mild symptoms of infection, which typically last for about a week and resolve without chronic symptoms. However, in individuals at higher risk, particularly infants, children, and adolescents, one or more symptoms may develop and can potentially lead to fatalities [18].

Common signs and symptoms of Enterovirus Infection are as follows:

- Common cold: runny nose, stuffy nose, cough, sore throat, mild fever, mild body aches
- Hypoxia or low blood oxygen: difficulty breathing, wheezing, coughing, rapid breathing, changes in skin color (blushing to cherry red), fast heart rate
- Aseptic meningitis: most common in infants and young children; May also occur with rash (on the face, neck, and extremities), fever, headache, neck stiffness, body aches, sensitivity to light, nausea and vomiting, and malaise.
- Conjunctivitis: eye pain, bleeding from the whites of the eyes, photophobia (avoiding light due to discomfort)
- Pericarditis: difficulty breathing, chest pain, fever, weakness

- Herpangina: small sores with a fluid-filled center with a red surrounding border on the oral mucosa (tonsils and soft palate) that can produce blisters and ulcers
- Angina: intermittent severe chest pain, usually in the lower part of the chest: Pain in the involved muscles is common.
- Hand, foot and mouth disease (HFMD): small, soft, gray blisters appear on the hands, feet and in the mouth
- Encephalitis: symptoms range from lethargy and drowsiness to personality changes, seizures and coma
- Paralysis (uncommon in both polio and non- polio viral infections): flaccid paralysis is often asymmetrical to the muscles of the affected extremity: lower limbs are more commonly affected than upper limbs (poliovirus, Enterovirus 71 and Coxsackievirus A7); Other non-polio Enteroviruses often have less severe symptoms such as muscle weakness or ophthalmoplegia if paralyzed.

Some strains of Enterovirus produce different symptoms, some of which are more severe than others. Enterovirus D68 and Enterovirus 71 cause more severe neurological symptoms and disease[18].

1.4.2. Epidemiology of enterovirus infection

In Southeast Asian countries, the most important and widespread enterovirus pathogen is *Enterovirus 71*. Enterovirus 71 is a neurotropic virus known to cause severe and even fatal disease in children. In 1998, a large outbreak of enterovirus infection occurred in Taiwan. According to reports, 405 severe cases were reported among children, 78 of which were fatal. Of the 78 reported deaths, 71 children (91%) were under 5 years of age. The leading cause of death was EVA71 infection. Most of the examined patients died within 1-2 days of hospital admission. This led researchers to hypothesize that EVA71 directly affects the central nervous system, causing neurological pulmonary edema and myocardial dysfunction through mechanisms involving increased sympathetic activity and inflammatory responses. Early detection of these risk factors, along with proactive treatment, were critical measures for the successful management of the outbreak in Taiwan. In addition,

EVA71 has been shown to be a virus with devastating effects on children. Therefore, vaccines against this pathogen have been developed. Vaccination is considered the most effective measure in combating this virus [75].

According to the WHO report for the period 2010-2014, China accounted for 9.8-11.3 million of the total reported cases of hand, foot, and mouth disease, accounting for 87%. Enterovirus71 has been proven to be the main cause of severe HFMD. From 2011 to 2021, a total of 621 cases of HFMD were reported in Shanghai. However, there were no severe cases or deaths. The average age at onset was calculated to be 4.1 years, and the median age was 3.7 years. The youngest case was 2 months old, and the oldest was 26 years old. In this study, the sex ratio was 1.45:1.0, specifically, 368 males (59.3%) and 253 females (40.7%) were affected. Among the patients, 290 (46.7%) were preschoolers, 257 (41.4%) were schoolchildren, 73 (11.8%) were students, and only 1 (0.1%) patient was an adult. Regarding immunization against EVA71, only 72 people (11.6%) were vaccinated, and 134 people (21.6%) had no information about vaccination [139].

According to a study in southern Vietnam, hand, foot, and mouth disease (HFMD) has been identified as a common enterovirus infection, not only in the country but also the most prevalent in Southeast Asia. The first cases of hand-foot-and-mouth disease complicated by encephalitis were reported during an encephalitis outbreak in 2003 when enterovirus EVA71 was isolated by researchers of the Pasteur Institute in Ho Chi Minh City as one of the etiological agents of the disease [114]. Since the beginning of 2018, the number of hospitalizations due to hand, foot and mouth disease has increased significantly across Vietnam [46]. More than 53,000 clinical cases have been reported, including 06 deaths as of the end of September. This disease can be caused by many different serotypes of EV A species, of which EVA71, CVA6, CVA10, and CVA16 were the most frequently detected serotypes [2, 67, 128]. Among these serotypes, EVA71 is the serotype that causes the greatest burden due to the large number of hospitalizations and a relatively large proportion of severe illnesses and deaths. EV-A71 exists as a single serotype, but is genetically

divided into multiple genotypes (including A, B, and C) and subtypes (e.g., B1 to B5 and C1 to C5) [122]. Although there is no genetic evidence for differences in virulence between different genotypes, subgenotype replacement is often accompanied by large outbreaks of severe HFMD. Notably, the switch from C5 to C4 in 2011 coincided with an explosive outbreak in Vietnam, leading to more than 200,000 hospitalizations and more than 200 deaths from 2011 to 2012 [67].

1.5. Methods for diagnosing poliomyelitis and enterovirus infection

1.5.1. Methods for diagnosing poliomyelitis

The role of diagnostics in polio eradication efforts cannot be overstated. In countries such as Vietnam, where wild polioviruses no longer circulate, laboratory surveillance provides timely and accurate information on possible importation of wild poliovirus, so that its spread can be interrupted in a timely manner and virological evidence can be provided to certify polio eradication.

In the primary diagnosis of acute flaccid paralysis (AFP), poliovirus isolation from faecal samples is mandatory using two cell lines certified for this purpose. Positive isolates are identified using diagnostic sera in a micro neutralization test on the same cell lines. All poliovirus strains must undergo intratypic differentiation using real-time PCR (rRT-PCR_ITD/VDPV) to determine whether the strain is wild-type or of vaccine origin. The classical method of virus isolation on cell cultures, being the gold standard, is expensive in terms of time and human resources. As an alternative, molecular biology methods (RT-PCR) have been developed that detect poliovirus directly in the sample of the material being studied. Direct extraction of polioviruses or their fragments, according to the World Health Organization, is promising for widespread use in the future. Serological methods, testing for the presence of antibodies that neutralize the polio virus, are generally not recommended for routine use in diagnosing polio because of the high space and time requirements, difficulties in analyzing the results, and the method does not distinguish between antibodies to the "wild" type of the virus and vaccine strains.

Testing methods for poliovirus recommended by WHO include:

(1) Method of culturing polioviruses and other viruses on RD and L20B cell lines;

(2) Determination of the poliovirus type by the micro neutralization method;

(3) Determination of poliovirus type, determination of whether it belongs to the "wild" type or vaccine-associated type by the RT-PCR method (ITD/VDPV)[78].

Cases in which patients show no symptoms of central nervous system involvement or minimal or no symptoms are often not diagnosed by doctors if they occur in a non-endemic country where there is no evidence of wild poliovirus circulation.

In children or adolescents who are not vaccinated against polio, paralytic disease may develop, which is characterized by paralysis of the limbs or spinal cord damage without sensory disturbances during the acute febrile phase. There are non-polio enteroviruses that, like polioviruses, can cause acute flaccid paralysis of the upper or lower limbs in children. Most often, Coxsackie viruses A and B (especially A2 and A7), some echoviruses and enterovirus 71 can cause similar symptoms. With a similar clinical picture of such flaccid paralysis, residual paralysis, as in poliomyelitis, rarely develops. The preliminary diagnosis in these cases is also AFP and the course of laboratory testing is similar and enteroviruses of different types can be isolated on the cells.

In addition, cases have been reported with weakness or paralysis of the limbs following infection with enterovirus EVD68. Clinically, acute flaccid paralysis caused by West Nile virus may resemble poliomyelitis, making it difficult to differentiate between the two. On the other hand, Guillain-Barré syndrome may also cause flaccid paralysis, but it can be distinguished by the following characteristic features:

- The disease usually does not cause fever.
- Symmetrical muscle weakness.
- Sensory disturbances occur in 70% of patients
- Protein in the cerebrospinal fluid is often elevated, but cytosis is normal.

If the patient has stiff neck, a diagnosis of viral (aseptic) meningitis is assumed. In these cases, patients with meningitis undergo a lumbar puncture, in the cerebrospinal fluid (CSF) a normal glucose level, an elevated protein level, cytosis (usually lymphocytosis) from 10 to 500 microliters are usually determined.

When determining the cause of a disease with neurological symptoms, epidemiological data including age, season, vaccination history, and recent travel history are extremely important factors in helping to establish the etiology of the disease.

1.5.2. Testing methods for enterovirus infection

Samples for virological and molecular biological studies [37] include feces, throat swabs, vesicular fluid, ulcerative fluid, and cerebrospinal fluid.

Titers of type-specific antibodies are determined in the blood serum.

Diagnostic methods such as virus isolation by inoculation of a sample into monkey kidney cells, human cells or other sensitive lines are used. The cytopathogenic effect of viruses is determined.

RT-PCR detects viral RNA. Neutralization or immunofluorescence reactions allow determination of type-specific antibody titers. Due to cross-reactivity between different types of enteroviruses and the duration of the method, serological reactions in the diagnosis of EVI are used for specific purposes [37]. For example, to confirm the etiologic role of poliovirus or enterovirus of a certain type. In the neutralization reaction, paired patient sera are examined to detect a fourfold increase in antibody titers to the corresponding virus.

1.6. Importance of the Polio Eradication Program for Improving Enterovirus Surveillance

In 1980, significant progress was made in smallpox eradication [29] and poliomyelitis from the Americas [22]. In 1988, the 41st World Health Assembly (WHA) committed to the “global eradication of poliomyelitis by the year 2000”. The Global Polio Eradication Initiative (GPEI) was launched. WHO and partners UNICEF, Rotary International, and the US CDC have been largely successful in

reducing the incidence of poliomyelitis. National polio control programs are critical to eliminating the infection [39].

For example, in England and Wales, polio eradication was achieved primarily through childhood and adult vaccination programs. Live oral poliovirus vaccine and inactivated poliovirus vaccine (OPV and IPV) were used. IPV was first introduced and rolled out in 1956, but was replaced by OPV in 1962 and became part of the routine vaccination program. By 2004, OPV had been replaced by IPV due to the risk of vaccine-associated poliomyelitis (VAPP) in recipients of the live vaccine [61]. Poliovirus surveillance has become increasingly complex due to the diversity of methods and the ongoing risk of polio re-emergence. Polio surveillance in England and Wales has required adaptation over the decades between endemicity and eradication. In the latter stages of eradication, monitoring of poliovirus circulation remains critical [97].

In Germany, the last indigenous polio case was in 1990. Then in 1998, Germany switched from using the oral poliovirus vaccine to IPV in its vaccination program. That same year, the National AFP Surveillance Agency was established in Germany. The goal was to establish a national surveillance system for patients with acute flaccid paralysis syndrome and to test a variety of clinical specimens (faeces, respiratory tract swabs, cerebrospinal fluid) to improve the ability to detect enteroviruses that cause serious disease [66]. Therefore, Germany decided to establish the Laboratory Network for Enterovirus Surveillance (LaNED) [9], based on the National Enterovirus Surveillance System (NESS) in the United States. NESS is a voluntary, passive surveillance system that has been monitoring the detection of enteroviruses and parechoviruses in laboratories since the 1960s [1, 10].

In conjunction with the Global Polio Eradication Initiative, the National Polio Eradication Program was adopted in Russia in 1996. In addition to AFP surveillance, the country also introduced wastewater surveillance as a mandatory part of poliovirus circulation surveillance [56]. Virological studies of wastewater have been conducted since the mid-20th century to assess the quality of wastewater treatment and to obtain

information on circulating enteroviruses. Wastewater monitoring is of particular importance, especially at the end of the program, and is certainly an important part of poliovirus surveillance [3, 26, 48].The wastewater monitoring methodology in Russia is based on the recommendations of the World Health Organization (WHO) [35] and national guidelines [56], and is aimed primarily at identifying polioviruses.

India has been a polio-burden country since the early 1990s, with 500-1000 children becoming infected daily. Despite the introduction of OPV through the Expanded Program on Immunization (EPI) in 1979, the polio burden did not decrease for many years. It was only through extraordinary efforts that transmission of wild poliovirus was interrupted in the country. Since 2004, annual polio vaccination campaigns have been conducted 10 times a year. The campaigns not only included vaccination but also monitoring of virtually every child. As a next step, India has entered Phase 2 with the introduction of IPV, the switch from tOPV to bOPV, and the ultimate goal of eliminating all vaccine-derived polioviruses and polioviruses vaccines [62]. The country signed the 1988 WHA resolution, which called for the eradication of polio worldwide by the year 2000 [39]. Achieving and maintaining high levels of routine OPV vaccination coverage, conducting supplementary immunization activities (SIA), establishing systematic polio surveillance with support from virology laboratories, and using local mop-up immunization campaigns to break any remaining chains of WPV transmission were the four strategic components proposed by WHO [12].

In Vietnam, before the advent of vaccines, polio was a leading cause of death among children under 5 years of age, with many children who recovered from the disease suffering long-term consequences. Faced with this situation, the Ministry of Health included the oral polio vaccine in the expanded immunization program: the first dose is given to children at 2 months of age, the second dose at 3 months of age, and the third dose at 4 months of age.

There are several approaches available for characterizing and detecting enteroviruses, such as poliovirus, including general protocols that have been

developed and advised by the WHO and the CDC, respectively. Thus, the collection of samples should be the main focus of efforts to strengthen the system. Initially, by incorporating regular monitoring of respiratory samples from kids seen in both primary and secondary healthcare settings. Second, by guaranteeing that patients with severe unexplained respiratory disease and/or unexplained neurological clinical presentations have their stool, cerebrospinal fluid, blood, and respiratory samples diagnostically analyzed. Thirdly, by incorporating surveillance of the environment. In the post-polio era, the latter system is the primary methodological suggestion for enterovirus surveillance [45].

Since 1988, when the global polio eradication initiative was launched by WHO, using four primary strategies, this effort has nearly eradicated the disease brought on by wild poliovirus strains: First, by coordinating worldwide systems Acute and systematic monitoring of flaccid paralysis screening stool samples from acutely ill patients paralyze; Second, through word screening samples Patients with aseptic or environmental meningitis Polio virus surveillance or both in many countries with a low incidence of acute flaccid paralysis; third, through Actively campaign for universal vaccination to cover vaccination rates; and finally, through targeted vaccination during disease outbreaks for prevention spread [142] .

Although there are still many challenges in the surveillance system, the establishment of surveillance programs and platforms such as those described above will help to raise awareness of polio worldwide and of circulating enteroviruses and their ability to cause disease [30]. This is critical to monitoring the incidence of EVI. Vietnam has eradicated polio since 2000 and is currently implementing the Expanded Programme on Immunization, which contributes to protecting Vietnamese children from polio. In addition to the Expanded Programme on Immunization, it is necessary to promote good hygiene and personal hygiene and educate the community on these issues. Polioviruses are transmitted by the mouth and intestines, so it is important to keep water sources clean and ensure food hygiene and safety [33].

Since June 2016, Vietnam has been vaccinating all children aged 2, 3 and 4 months with the oral poliovirus vaccine bOPV instead of tOPV. Since September

2018, Vietnam has added a fourth dose of vaccination for children at 5 months of age with inactivated poliovirus vaccine. On 27 February 2023, WHO and UNICEF made a request under the Expanded Program on Immunization to Vietnam to increase the number of polio vaccinations for unvaccinated or incompletely vaccinated children to the full immunization course for such children in order to prevent the occurrence of epidemics in Vietnam [144].

CONCLUSION

Moreover 50 years of research on enteroviruses has been accompanied by changing views on their properties and importance in human infectious pathology. According to the latest update, the genus *Enterovirus* belongs to the family *Picornaviridae*, according to traditional classification, divided into 4 subgroups, including: Poliovirus, Coxsackievirus A, Coxsackie B, Echovirus [92]. Within the species, types of enteroviruses were identified. Currently, more than 100 representatives of the genus are known *Enterovirus*, causing a wide range of epidemically significant diseases that vary in clinical manifestations and severity (from mild forms of respiratory and intestinal infections to hand-foot-and-mouth disease manifestations and severe neurological symptoms and even life-threatening multiple organ failure). In the medical literature, enteroviruses Coxsackie A16 and enterovirus 71, which cause HFMD, are often mentioned as actively circulating among the population of different countries. In the countries of Southeast Asia, cases of this infection are widespread and are often complicated by severe neurological symptoms.

Given the etiological diversity of EVI and the differences in clinical manifestations, diagnostics is based on laboratory methods for identifying pathogens. The "gold standard" of the virological method is the isolation of pathogens with subsequent typing in the neutralization reaction. Recently, molecular methods of laboratory diagnostics of EVI have been developed. Improvements of the laboratory component of epidemiological surveillance of EVI is possible only with the integrated use of classical and modern research methods.

The prevalence of EVI pathogens, the unstable epidemic situation worldwide and the processes of active population migration create a constant threat of importation and spread of EVI. For example, wild polioviruses have been imported from Nigeria to 26 other African countries. In this way, international spread of wild polioviruses from endemic countries to poliomyelitis-free countries can occur.

In 1988, the World Health Assembly resolved to eradicate polio. Combined with intensive vaccination program, clinical and virological surveillance based on acute flaccid paralysis cases have proven remarkably effective, even in high polio burden countries. Since WPV3 was last detected in Nigeria in November 2012, WPV1 is the only wild poliovirus that continues to circulate in the two endemic countries where WPV1 transmission persists: Afghanistan and Pakistan. In August 2016, WPV1 was also detected in Nigeria [143] and the potential for localized transmission still exists. The Polio Eradication Strategy 2022–2026 expected to interrupt WPV1 transmission by the end of 2023 [103], but WPV1 continues to cause paralytic polio in two endemic countries in 2024 [123]. Achieving the challenging goal of polio eradication requires an unwavering commitment to universal IPV vaccination coverage worldwide. A variety of surveillance strategies are being used to achieve full vaccination coverage in areas where OPV is used.

Despite the successes achieved, a number of unresolved problems in the fight against infections remain to this day: establishing patterns in the development of epidemic increases in EVI incidence; the reasons for the emergence and circulation of altered strains of enteroviruses that cause seasonal increases in EVI; the molecular genetic characteristics of circulating enteroviruses and their phylogenetic relationship with pathogens circulating in different territories of countries and the world are insufficiently covered.

All of the above, as well as the high socio-economic significance of the problem of morbidity and mass outbreaks of enterovirus infection caused by enteroviruses of various types, require further improvement of virological surveillance of this infection and further improvement of anti-epidemic measures to prevent mass morbidity from EVI.

MATERIALS AND METHODS

The research was held according to standard WHO procedures (Polio laboratory manual, World Health Organization: WHO) [78] at Enteroviruses laboratory (Immunology and microbiology department), which is the National Polio Laboratory for South Vietnam. The laboratory is part of the World Health Organization network of polio laboratories annually accredited by WHO based on performance of a professional test. It participates in the implementation of WHO Program of Global eradication of poliomyelitis and the National Guidelines for surveillance and prevention of hand, foot, and mouth disease created by Ministry of Health VietNam.

The research approved by the Institutional Ethics committee in biomedical research of Pasteur Institute in Ho Chi Minh City No 18/CN-HĐĐĐ dated 10/12/2019 and the Independent Ethics Committee of Institute Pasteur Saint Petersburg No. 54 dated 29/10/2019) was conducted from 2022 to 2024.

The study is based on the results of epidemiological and virological surveillance of cases of acute flaccid paralysis (AFP) of poliovirus and non-poliovirus etiology in 29 provinces of Vietnam in accordance with the WHO recommendations for the poliomyelitis eradication program. In addition, a study was conducted within the framework of the national program for surveillance and virological diagnostics of various clinical forms of enterovirus infection in 20 provinces of South Vietnam, including diseases such as viral exanthema of the oral cavity and extremities, as well as other neurological diseases: enterovirus meningitis/meningoencephalitis (Figure 3).

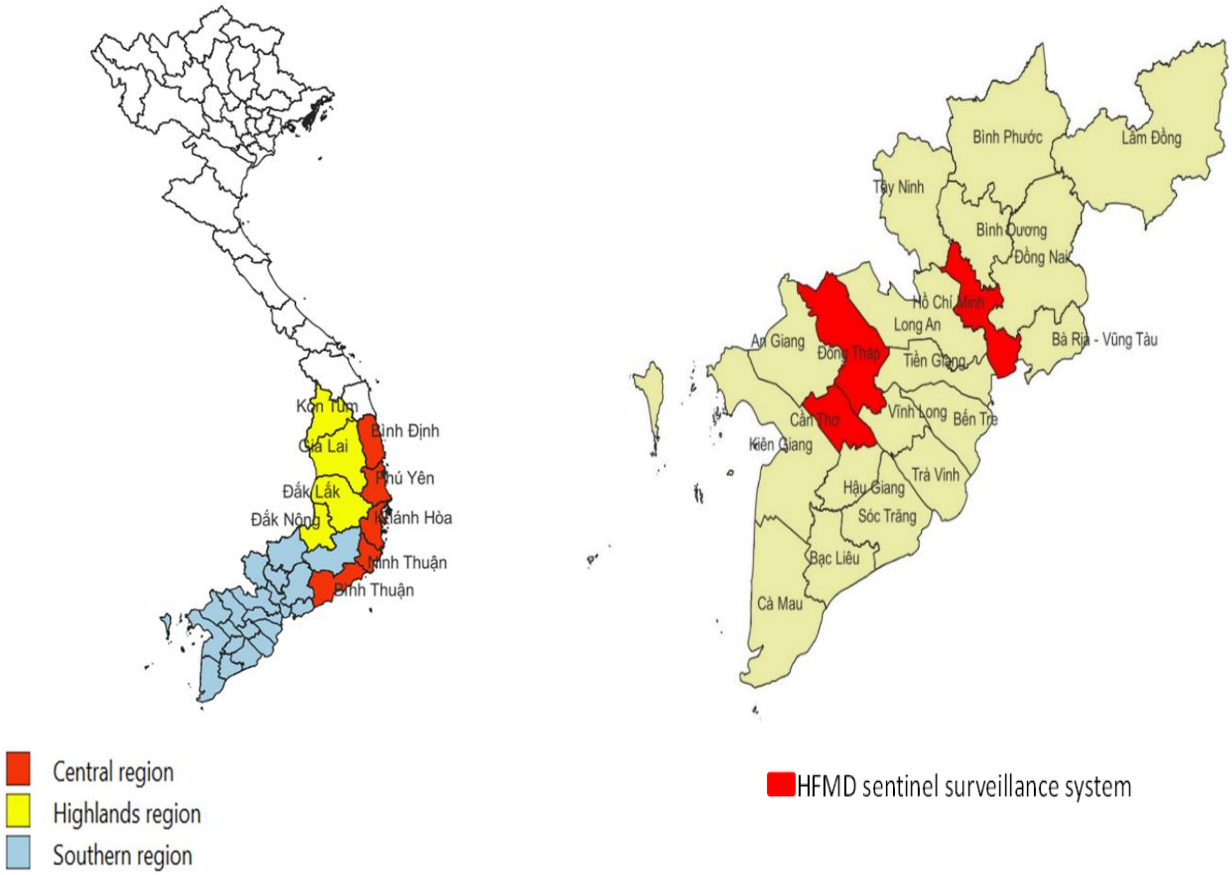

Figure 3. A. 29 provinces of AFP surveillance B. 20 provinces of enterovirus surveillance in South Vietnam

2.1. Research materials

The study participants were patients aged ≤15 years old in South Vietnam admitted to pediatric departments with primary diagnoses of acute flaccid paralysis, hand, foot, and mouth disease (HFMD) meningitis, meningoencephalitis, and enterovirus infection with other symptoms. The study was conducted by decision of the Ministry of Health in accordance with the guidelines for surveillance of acute flaccid paralysis and monitoring of hand, foot, and mouth disease (HFMD). Good quality samples from all these patients were sent to the Pasteur Institute in Ho Chi Minh City for testing. Samples from AFP patients were collected between 2010 and 2021, and from enterovirus infection patients between 2012 and 2021.

The following information about each case was used to analyze the incidence of enterovirus infection and the etiologic agents of the diseases: age, sex, address, date of onset of the disease, date of sampling, and clinical symptoms. The cases were divided into three categories: acute flaccid paralysis; hand, foot and mouth disease;

aseptic meningitis, meningoencephalitis, encephalitis (encephalopathy).

Diagnostic criteria for infections.

Criteria for cases.

Hand foot and mouth disease

- Fever ≥ 38.5oC
- Sore throat
- Mouth ulcers at anterior buccal and gingival mucous membranes (gum) and tongue.

And/or rash on palms, soles, knees, buttocks (rash with vesicular, maculopapular, papulovesicular or petechial forms, morbilliform exanthems, with diameter of 1-3 mm).

Aseptic meningitis

- Temperature ≥ 38.5◦C
- Vomit
- Meningeal symptoms: neck rigidity, Kernig's sign (+)
- Improvement of clinical condition without the use of antibiotics.
- And/or the CSF appears clear and the white blood cell count is low to moderate (10-500/ml).

Encephalitis:

- Temperature ≥ 38.5◦C
- Disturbances of consciousness (lethargy/ drowsiness/ confusion/ loss of consciousness / coma).
- Cramps

Meningoencephalitis:

- Symptoms of encephalitis plus meningeal signs

Acute flaccid paralysis (acute flaccid paralysis syndrome):

- Temperature ≥ 38.5◦C

Sudden onset flaccid (often asymmetrical) paralysis

- Loss or decrease in motor activity of the nervous system
- No loss of sensitivity

Acute flaccid paralysis syndrome includes a number of paralytic diseases (Guillain-

Barré syndrome, Landry's paralysis and other flaccid paralyses).

All surveillance activities require laboratory testing of each case and must be accompanied by good quality specimens. After collection, specimens were refrigerated if they arrived at the laboratory within 72 hours of collection. Otherwise, specimens were frozen at -20°C and shipped frozen.

Two stool samples were collected from patients with AFP, 24–48 hours apart, for 14 days after the onset of paralysis. If spontaneous defecation is not possible, a rectal swab should be taken for testing.

Patients with HFMD or other neurological diseases had a single stool sample (rectal swab) and/or throat swab collected within 7 days of illness onset. The material was tested to determine whether they were infected with enteroviruses. Stool samples (4–8 grams) were collected in a vial, and all swabs were placed in a tube with transport medium.

The effectiveness of virological diagnostics depends on the correct and timely collection of clinical samples, as well as on the observance of optimal conditions for their transportation to the laboratory (cold chain principle) and ensuring biological safety. All procedures were carried out in accordance with WHO recommendations, the requirements of the Laboratory Biosafety Manual (LBM) and the instructions for the collection, transfer and transportation of samples of the Department of Preventive Medicine of the Ministry of Health of Vietnam.

All isolated polioviruses were sent to the Laboratory at the national Institute of infectious diseases Japan (NIID Japan) for intratypic differentiation poliovirus and sequencing to identify the type of poliovirus.

The total number of samples from various sources examined in laboratory of Pasteur Institute in Ho Chi Minh City for 10 years is presented in table 2.

Table 2

Number of cases examined in the laboratory enterovirus of PI HCMC in 2010-2021

Sample source	**Number of samples**		
	Stool	Throat swabs	Total
a primary diagnosis of acute flaccid paralysis	2.143	0	2.143
a primary diagnosis of encephalitis, aseptic meningitis	0	1.004	1.004
a primary diagnosis of Hand-foot-and-mouth disease	1.186	6.790	7.976
Total			**11.123**

All samples from patients with a primary diagnosis of acute flaccid paralysis, hand-foot-and-mouth disease or neurological disease (encephalitis, aseptic meningitis) were studied only in laboratory enterovirus of PI HCMC, the National Laboratory in Vietnam. Analysis of the annual documentation was performed in order to confirm the polio-free status of South Vietnam in 2010-2021.

Summarized data on the isolation and molecular identification of polioviruses and non-polio enteroviruses of various types from patients with enterovirus infection were the following. When examined 2.143 fecal samples from children with AFP syndrome 22 polioviruses of three types (1.0%) were isolated and identified. From 8.980 studied samples from patients with EVI, 6.180 enteroviruses of various types were identified. Sequencing of full or partial of VP1 genome region of strains or clinical samples was carried out in the laboratory to determine the etiology of viral infection of examined patients with different clinical forms of enteroviral infection.

2.2. Research methods

Scheme of analysis of samples from patients with acute flacid paralysis syndrome viral meningitis /encephalitis or HFMD is shown in Figure 4.

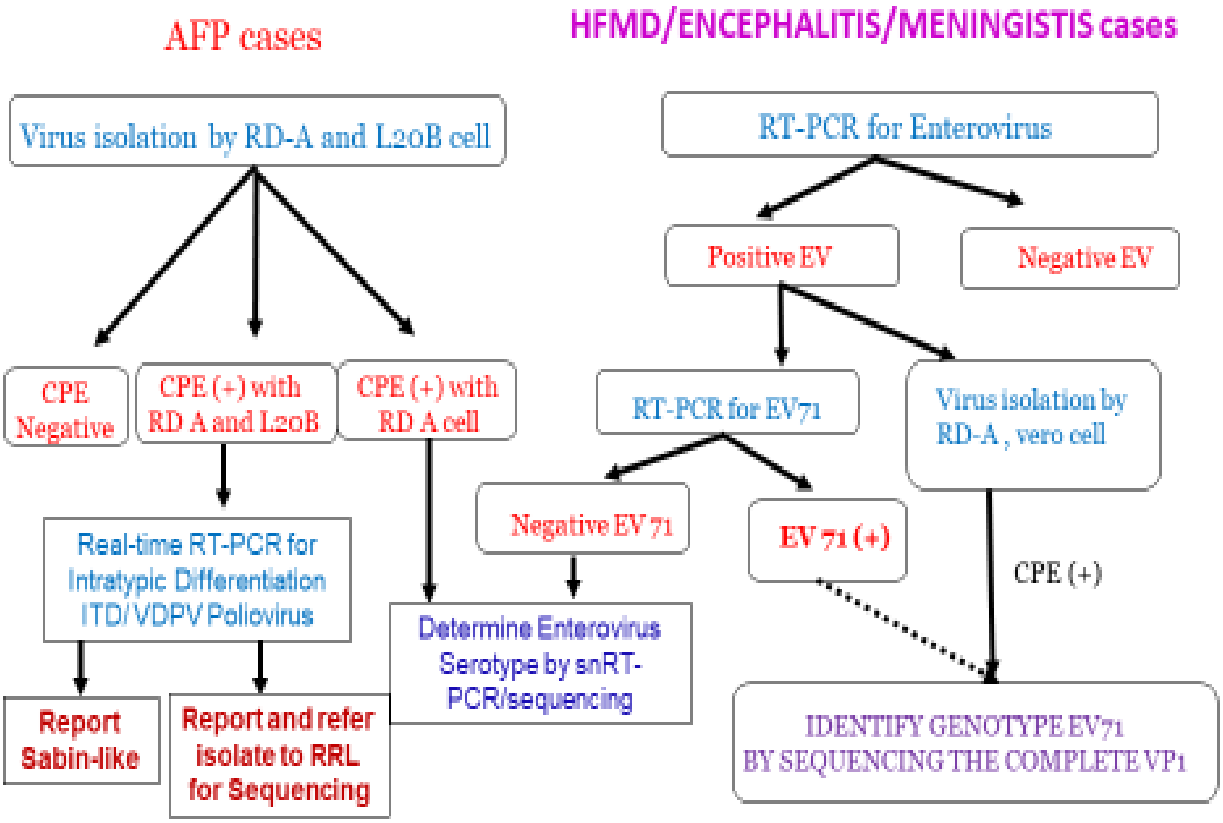

Figure 4. Scheme of analysis of samples from patients with AFP and enterovirus infection.

Sample's treatment was carried out in accordance with WHO recommendations (WHO Polio laboratory manual, 2004) [78] and the guide to clinical management and public health response for hand, foot and mouth disease (HFMD) by World Health Organization. Regional Office for the Western Pacific 2011) [34].

Stool specimen was initially processed with 10% chloroform in phosphate buffered saline (PBS) containing $MgCl_2$ and $CaCl_2$ with a solution of antibiotics. Chloroform removes bacteria, fungi, cytotoxic lipids, dissociate viral aggregates. Enteroviruses are not sensitive to chloroform. Two grams of sample were mixed with 9 ml of PBS, 1 ml of chloroform and one gram of glass beads were added, shaking vigorously for 15 min and centrifuged for 20 min at 1500g in a refrigerated centrifuge.

Throat swabs or rectal swabs were placed in a tube with virus transport medium. Throat/rectal swabs were vortexed quickly and cotton stick removed before

centrifuging for 15 min at 5.9 g. The supernatants from both stool and throat swabs were stored in -20^{0}C until virus identification procedures were performed.

2.2.1. Isolation of enteroviruses

Isolation of polioviruses were performed on two cell cultures: RD and L20B according to algorithm recommended by WHO.

For isolation and identify enteroviruses from patients with EVI or neurological disease, all EV- positive samples obtained by RT-PCR were inoculated onto RD cells (human rhabdomyosarcoma cells) and Vero cells (the kidney of an African green monkey cells). These cell lines were used because of their relatively high sensitivity and the apparent cytopathic effect (CPE) induced by EV71 and other enteroviruses.

For exclusion of polioviruses, all samples which showed signs of cytopathogenic effect on one or both Vero or RD cell culture, were passaged on cell culture L20B (modified mouse fibroblasts having on their surface receptors for polio viruses) that exhibit specific CPE when infected with poliovirus.

Virus isolation was performed with at least two passages for each specimen. All cell lines are available from the Global Polio Laboratory Network and the quality control of the cells is routinely carried out according to the Polio Laboratory Manual to ensure sensitivity against polioviruses. The quality of cell cultures used in virological investigation is important for the standardization of enterovirus isolation and its characterization.

The cells were cultured in a nutrient medium EMEM for L20 B and RD cell and DMEM for Vero cell with double a set of amino acids with the addition of 7% fetal bovine serum and antibiotic solution. An antibiotic solution was added at the rate of 100 IU of penicillin and 100 mcg streptomycin in 1 ml of nutrient medium. Cells were grown in plastic tubes with 1 ml maintenance medium. For studies of one sample used 1 vial of each culture for 2 tubes per cell line, one tube with each cell type was left uncontaminated as a negative control. Each tube was inoculated with 0.2 ml of suspension containing specimen and incubated at 36°C in a thermostat (at 5% CO2). The content of non-polio enterovirus in the sample was determined by the characteristic CPE: rounding of the cells and their detachment from the growth layer.

The cell culture was examined daily under an inverted microscope for five days until there was no evidence of cytopathogenic effect or until CPE had spread to 75-100% of the cells. If CPE was not present, the tubes were frozen at -20°C, 0.2 ml of culture fluid was then passage into tubes containing fresh cells of the same cell type, and the sample was monitored daily for five days. If CPE was not present at this point, the result was considered negative. If one or both cultures showed signs of CPE, an additional passage was performed on the L20B cell line to exclude poliovirus. After freezing, the culture fluid was removed from the vial containing the cells for storage at -20°C.

2.2.2. Molecular biological method

Molecular identification was used to identify isolated strains of polioviruses and non-polio enteroviruses. Viral RNA was isolated from 140 µl of fecal supernatant or oropharyngeal /rectal swabs using the QIAamp extraction kit according to the manufacturer's instructions (QIAGEN).

2.2.3. Identification of EV by pan Enterovirus RT-PCR

A pan-Enterovirus RT-PCR (reverse-transcription polymerase chain reaction) was applied for screening presence of enterovirus in specimen using a primer set that flanks the conserved nucleotide sequence in the 5′UTR of the enterovirus genome. The sense primer (MD90) has the sequence 5’-ATT GTC ACC ATA AGC AGC CA-3’, and the sequence of antisense primer (MD91) sequence is 5’-CTT CCG GCC CCT GAA TGC GGC TAA T-3’. These primers flank the target nucleic sequence 5’-UTR of EV genome with an expected product size of 154 bp.

a. RT reaction: 6 µl of the viral RNA template was added to 1 µl of sense primer and incubated at 70°C for 10 minutes then it was put on ice immediately to stop reformatting the secondary structure of the RNA template. RT master mix was prepared with 2 µl M-MLV RT 5X reaction buffer; 0.5 µl M-MLV RT (Promega, cat# M1701) and 0.5 µl of 10 mM dNTPs. A total volume of 3 µl RT master mix was added to 7 µl of the mixture of the previous step to make a final volume up to 10 µl.

This mixture was incubated at 42° C for 60 min then at 70°C to stop the reaction and it was cooled down on ice.

b. PCR reaction: 2 µl of 10X buffer in (NH4)2SO4 were added to 1.2 µl of 25 mM MgCl2; 2 µl of each primer of 10 pmol/µl; 0.6 µl of 10 mM dNTP, 2,5 U Taq DNA polymerase ((Promega Co., Madison, WI USA).); and sterile UHQ water to 10 µl of PCR master mix. Ten µl of PCR master mix were added to the above volume of RT reaction. PCR was processed as follows: 95°C/5 min for initial denaturation; 30 cycles of 95°C/1 min for denaturation of template; 60°C/1 min for annealing of primers to their complementary DNA (cDNA); 72°C/1 min for elongation of the cDNA. The final elongation was 1 cycle of 72°C/5 min.

c. Electrophoresis: The amplification products from the above PCR with approximately 154 bp were checked by electrophoresis parallel with molecular weight marker DNA GeneRuller 100 bp on 2% (w/v) agarose gel in TBE buffer and stained with Safe View.

2.2.4 Identification of EVA71 using EVA71-specific RT-PCR Screening Assays

All EV positive samples obtained by RT-PCR will have original specimens treated with chloroform and inoculated onto RD and Vero cell culture for virus isolation. The RNA extracts of these samples will be tested for EVA71 by EVA71-specific RT-PCR in parallel. The RT PCR for EVA71 was carried out with use of forward primer MAS01S of which sequence is 5'-ATA ATA GCA (C/T) T (A/G) GCG GCA GCC CA-3', and reverse primer (MAS02A) has the sequence 5'-AGA GGG AG (A/G) TCT ATC TC (C/T) CC-3'[101]. This primer set flanks the VP1 region of EV71 genome with product size of 376 bp.

a. RT reaction was applied as same as the one for EVs described above with antisense primer MAS02A.

b. In the PCR step, the VP1 gene of EVA71 was amplified by using 10 µL of cDNA in a 20 µL reaction volume containing 20 pmol each of forward primer MASO1S and

reverse primer MASO2A, 0.3 mM dNTP and 2.5 U Taq DNA polymerase (Promega Co., Madison, WI USA).

Amplification was performed with previously described at 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s to EVA71. The PCR products were examined by 2% agarose gel electrophoresis DNA staining by Safe View. Oligonucleotide primers for this assay, flank a region within the VP1 gene unique to EVA71 and amplify an expected product size of 376 bp.

2.2.5 Identification of subtype EVA71 by sequencing entire VP1 genomic region

All isolates with EVA71 identified by RT-PCR will be selected for nucleotide sequencing on the target genomic sequence (entire VP1 genomic region) of the viral cDNA (complementary deoxyribonucleic acid) to identify their genogroups and subgenogroups and compared with other EVA71 in the GeneBank for phylogenetic analysis.

Viral RNA was extracted from cell culture supernatant using the QIAamp viral RNA extraction kit (QIAGEN) and the VP1 gene amplified by reverse transcriptase-PCR (RT-PCR) using the Access RT-PCR System (Promega Co., Madison, WI USA). In brief, 2 µL of each viral RNA and 10 pmol of the forward primer 2349F (5'–GCYTAYATAATAGCAYTGGCGGCAGC-3') and the reverse primer 3393R (5'– GGCGGTTRACCACYCTDAAGTTGCCCAC-3') were used for each reaction mixture. The thermal and cycling conditions were as followed: 45 min at 480C, 2 min at 940C, 35 cycles of 940C for 10 sec, 500C for 10 sec, 650C for 1 min and then at 650C for 5 min. The presence of amplified fragments was confirmed by 1% agarose gel electrophoresis and the amplicons were purified with a Wizard SV gel and PCR clean up System (Promega). The entire VP1 genome of the purified amplicons was determined for both strands with oligonucleotide primers using an ABI 3130 genetic analyzer. The sequencing primers were, the 2349F, 2757F (sense: 5'–GCHAAYTGGGAYATAGACATAAC-3', H=A or T or C, Y=C or T), 2780R (antisense: 5'– CCCTRTATCTGTATTGDCC-3' R=A or G, D=A or G), and 3393R.

The complete VP1 gene sequences (891 nucleotides) for EVA71 strains were analysed by ABI 3130XL (Applied Biosystem).

Genetic analysis. The VP1 sequences were aligned using CLC Sequence Viewer 6, Mega 5, and were used to assign the strains to specific subgroups based on phylogenetic clustering with GenBank EVA71 reference strains. The VP1 sequence of the CAV16 prototype strain G10 was included as outgroup.

2.2.6 Identification of serotype EV using consensus-degenerate hybrid oligonucleotide primers (CODEHOP) RT-PCR targeting VP1 gene

All samples with cytopathic effect observed in cell culture were selected. The supernatant of cell culture with CPE induced by viral isolates were tested to identify Enterovirus type by technical improvement of RT-PCR methods based on the VP1 region enabled partial VP1 sequencing using consensus degenerate hybrid oligonucleotide primer (CODEHOP) with a high sensitivity and broader specificity for all known enterovirus types [117]. Enteroviruses can therefore be identified by VP1 sequences derived from the CODEHOP PCR products directly from clinical samples.

PCR amplifying the VP1 gene region for sequencing: RT-snPCR for other EV detection was performed with primer pairs AN 32-35 (cDNA); SO222-SO224 (PCR 1 – outer loop PCR); AN88-AN89 (PCR 2 – PCR inside) by thermal cycling: [cDNA] 500C/50 min and 700C/15 min; [PCR 1] 940C/2 min, performed 35 cycles including 940C/30 sec, 500C/30 sec, 720C/45 sec and 720C/5 min; [PCR 2] 940C/2 min, performed 35 cycles including 940C/30 s, 550C/30 s, 720C/45 s and 720C/5 min [93]. The RT-snPCR product was checked by 2% gel electrophoresis, DNA staining by Sefe View with lines typical of other EVs from 350-400bp. From the samples identified as positive, the RT-snPCR product was sequenced to determine the serotype of other EVs.

Purification of the RT-snPCR product: Use the Wizard SV Gel and PCR Clean Up System (Promega) biological kit to purify the RT-snPCR product that amplified the VP1 gene region of other EVs.

Gene sequencing: The purified RT-snPCR product was sequenced using the BigDye Terminator v3.1 Cycle Sequencing (Applied Biosystem) kit, using a primer pair shared with other EVs when amplifying the region. VP1 gene used in PCR 2 of RT-snPCR reaction is AN88-AN89. Carry out PCR Cycler Sequencing reaction according to thermal cycle: 960C/1 min; perform 35 cycles including 960C/10 seconds, 500C/5 seconds, 600C/4 minutes; and 400C/7 min. Purify PCR sequencing products using the QIAGEN biological kit. Nucleotide sequencing was read using an ABI 3130 XL sequencer (Applied Biosystem). Then collect raw data, read results, analyze with Finch TV, CLC Viewer 6, MEGA 7 software [69]. After obtaining the complete VP1 gene region nucleotide sequence, use BLAST tool of NCBI database to compare the relationship and similarity with the VP1 region sequence of other EV strains published on the database, thereby determining the serotypes of other EVs circulating in South Vietnam.

2.3. Statistical data processing

The average errors were determined, and the significance of statistical differences was evaluated using Student's t-test. Differences were considered statistically significant at 95% confidence interval (values of $p < 0.05$)

3. RESEARCH RESULTS

3.1 ETIOLOGICAL CHARACTERISTIC OF ACUTE FLACCID PARALYSIS SYNDROM IN SOUTH VIETNAM

3.1.1 Isolation of polioviruses from patients with acute flaccid paralysis in South Vietnam during 2010-2021

In 2000, Certification Commission of Polio Eradication Initiative (PEI) of World Health Organization accredited Vietnam as polio free country together with other countries of Western Pacific WHO Region. However, it is necessary to continue to perform increasing vaccination and acute flaccid paralysis surveillance in order to maintain polio free status of Vietnam until achieving Global Polio Eradication. The most effective way to found paralytic poliomyelitis is surveillance of Acute Flaccid Paralysis syndrome. In order to detect cases of acute flaccid paralysis, the Ministry of Health of Vietnam has established two National Laboratories (NL) for the diagnosis of poliomyelitis. One of these laboratories operates at the Pasteur Institute in Ho Chi Minh City. The laboratory staff carries out virological diagnostics of all cases of acute flaccid paralysis in children under 15 years of age in order to study the etiology of these diseases. From 2010 to 2021, stool samples from 2143 patients diagnosed with AFP syndrome were collected in the NL and tested according to the WHO algorithm for pathogen detection in 29 provinces of Vietnam (Southern Region: 20, Highland Region: 4 and Central Region: 5).

According to WHO recommendations and National EPI Guidelines, the annual standard for AFP case detection is at least one AFP case per 100,000 children under 15 years of age. The AFP detection rate in South Vietnam in 2010-2020 has always met the WHO target, only in 2021 during the COVID-19 pandemic, this rate dropped to 0.7. The average rate was 1.62, the highest rate was 2.7 in 2012, the next year it was 2.1, and the lowest (0.7) was in 2021 (Figure 5).

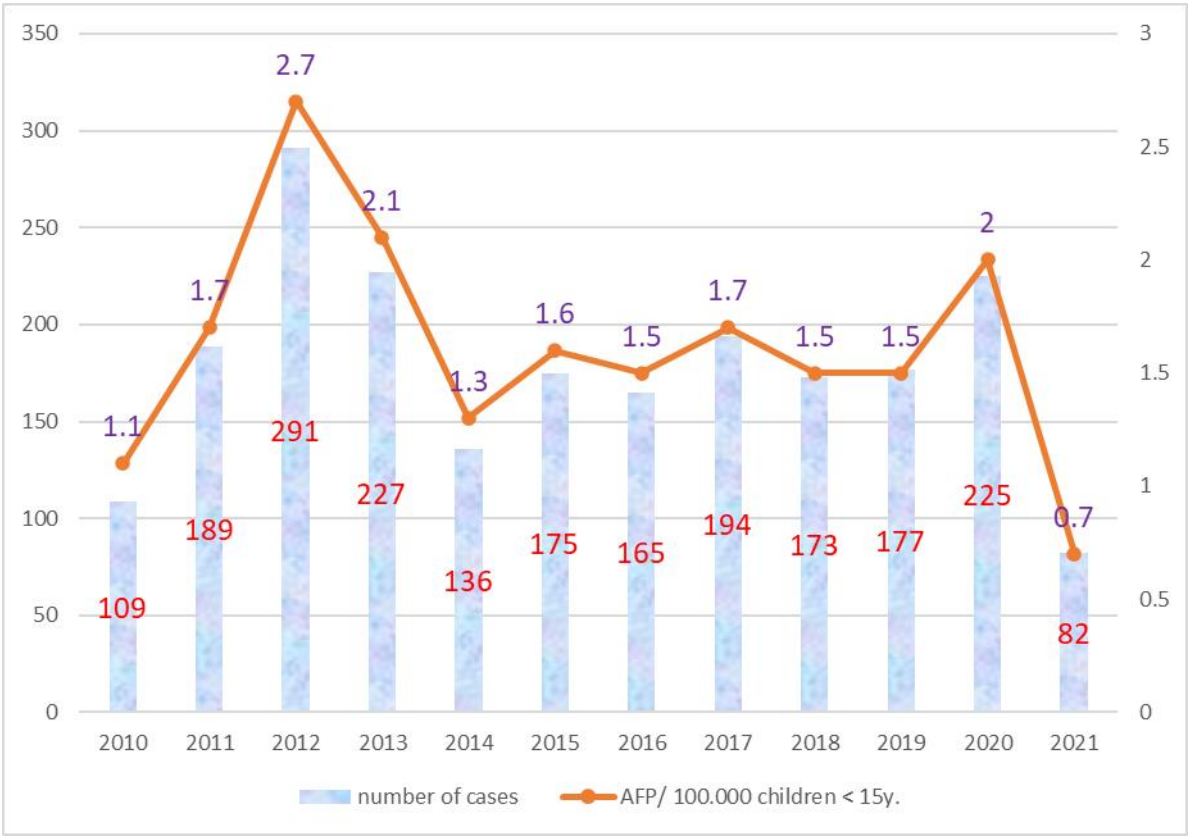

Figure 5. Number of AFP cases and AFP incidence rates in South Vietnam from 2010 to 2021.

In Vietnam, oral polio vaccine (OPV) has been deployed in the Expanded Program Immunization (EPI) since 1985. The domestically produced OPV vaccine includes 3 viruses types 1, 2 and 3 (tOPV). Vietnam has been polio-free since 2000. Vietnam continues to protect this achievement through effective acute flaccid paralysis surveillance and maintaining the coverage rate of three doses of polio vaccine among children at over 90%. Since 1993, the coverage rate of three doses of tOPV vaccine has always been above 90% and has been consistently maintained at a high level for many years. Since June 2016, Vietnam changed the oral poliovirus vaccine used from trivalent to bivalent (bOPV includes poliovirus types 1 and 3) for vaccinating children at 2, 3, 4 months of age. In September 2018, the inactivated poliovirus vaccine (IPV) was introduced and is given as a single dose to children at 5 months of age. However, due to the Covid epidemic, OPV vaccination coverage among children has dropped to 61.5% in 2020-2021 (Figure 6).

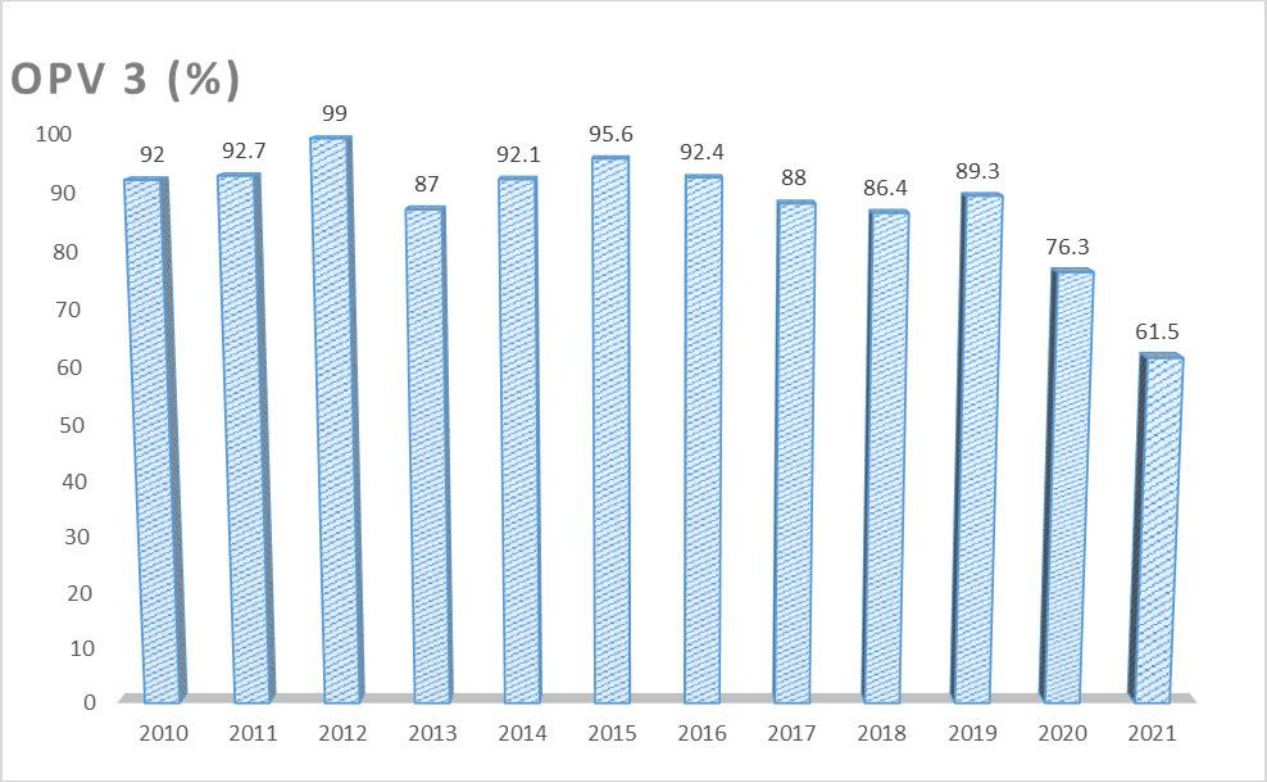

Figure 6. Percentage of children covered with three doses of polio vaccine (OPV3) in South Vietnam, 2010-2021.

During twelve years of surveillance for acute flaccid paralysis, the researchers of the National Polio laboratory in Ho Chi Minh City isolated 22 polioviruses from the 2.143 patients with primary diagnosis of acute flaccid paralysis syndrome.

The results showed that the percentage of poliovirus isolation in AFP patients was 1%. Most of isolated polioviruses were vaccine polioviruses according to the results of intratypic differentiation (ITD). Among 22 polioviruses, 20 strains were Sabin-like (SL) polioviruses and two isolated polioviruses were classified as vaccine-derived polioviruses of type 2 (VDPV2). Poliovirus identification showed that six polioviruses belonged to type 1, five polioviruses represented type 2 and eleven strains belonged to type 3 (Table 3). However, no wild poliovirus strains were isolated. It is important to note that the last PV2s were isolated in early 2016, before the global switch to bOPV vaccination instead of tOPV

Table 3

Poliovirus isolation from AFP cases in 2010-2021

Year	Number of AFP cases	Number of isolated polioviruses	Types of isolated polioviruses (PV)		
			PV1		
2010	109	1	-	-	1
2011	189	2	1	1	-
2012	291	5	2	2	1
2013	227	2	1	-	1
2014	136	-	-	-	-
2015	175	-	-	-	-
2016	165	8	1	2	5
2017	194	-	-	-	-
2018	173	4	1	-	3
2019	177	-	-	-	-
2020	225	-	-	-	-
2021	82	-	-	-	-
Total	2.143	22 (1%)	6	5	11

The ratio of different types among the 20 isolated poliovirus strains showed the following results: PV3 (11/20, 55%), PV1 (6/20, 30%), PV2 (3/20, 15%). Only seven children over 7 months old did not receive three doses of OPV, including two children infected with VDPV2 who were 18 months old and who were not vaccinated with OPV, and a five-year-old child received only 2 doses of OPV (Table 4). These two children live in areas with poor sanitation and low vaccination coverage, where spontaneous accumulation of mutations is possible during repeated transmission of polioviruses from vaccinated to unvaccinated children and the formation of virulent vaccine-derived viruses from vaccine-derived polioviruses. Further spread of such viruses can lead to infection of unvaccinated children with vaccine-derived poliovirus.

Table 4

Vaccination of children who are poliovirus isolates and diagnosed with AFP

Vaccination of children against polio		Number of children according to age					Isolated polioviruses
		0-6 m	7-12 m	13-36 m	3-14 y	Total	
Children vaccinated with OPV	3 doses	1	-	2	4	7	2 PVSL1, 1 PVSL2, 4 PVSL3.
	2 doses	4	4	-	1	9	1 VDPV2, 4 PVSL3, 2 PVSL1, 1 PVSL2+PVSL3, 1 PVSL1+PVSL3.
	1 dose	1	-	1	-	2	1 PVSL1, 1 PVSL3.
Non-vaccinated children		-	-	1	-	1	1 VDPV2
Total number of children		6	4	4	5	19	20 PVSL and 2 VDPV2

In April and June 2012, Vietnam declared two AFP cases from two provinces (Soc Trang and Dong Nai) in southern Vietnam and were classified as AFP cases associated with vaccine-derived poliovirus type 2 – VDPV2 (Figure 7).

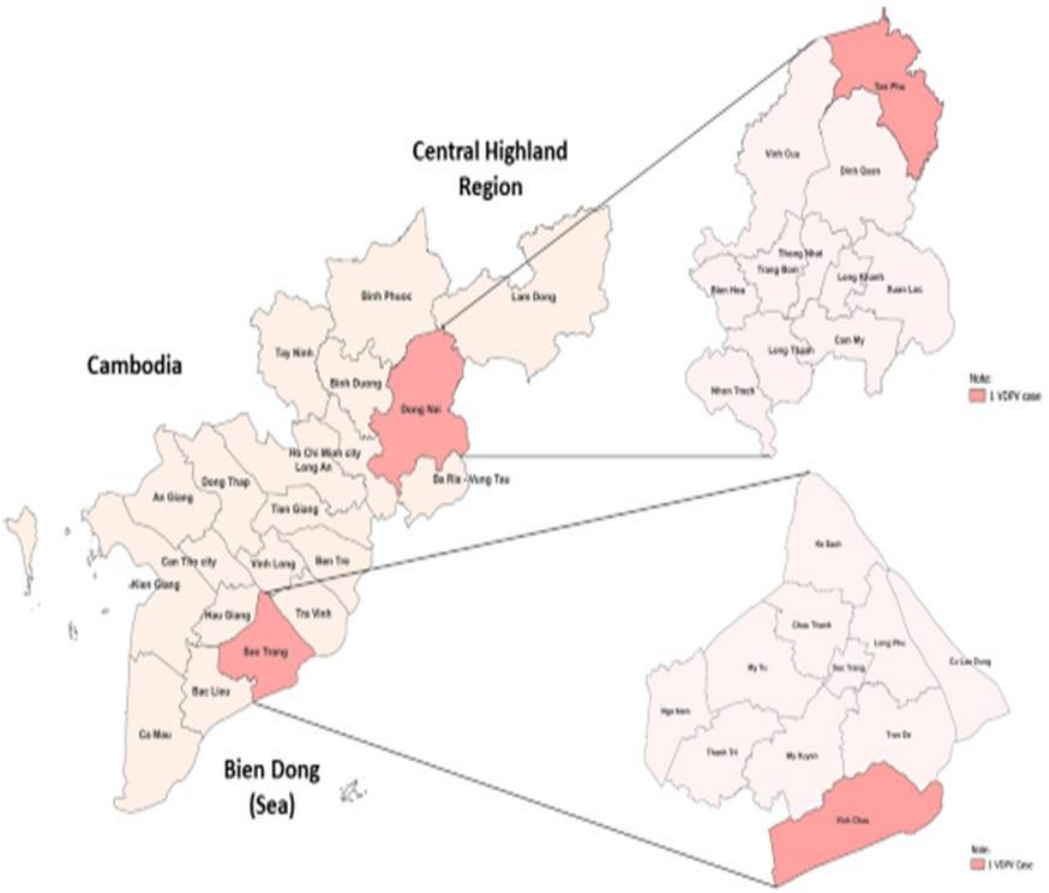

Figure 7. The map shows cases of AFP caused by PV type 2 registered in 2012 in two provinces of southern Vietnam.

The VDPV2 strains from these two AFP cases were studied by genome sequencing. Both polioviruses (SV3127LR-VP1 and SV3128LR-VP1), from the first child with AFP in Soc Trang province had six nucleotide substitutions in VP1 genome region. They were different from the reference strain of Sabin virus type 2 (0.66% divergence). The first poliovirus (SV3129LR-VP1) from the second child in Dong Nai province had five nucleotide substitutions. The second poliovirus (SV3130LR-VP1) isolated from the same child had a divergence percentage of 0.66%, since it had six nucleotide substitutions compared with Sabin virus type 2 (Figure 8).

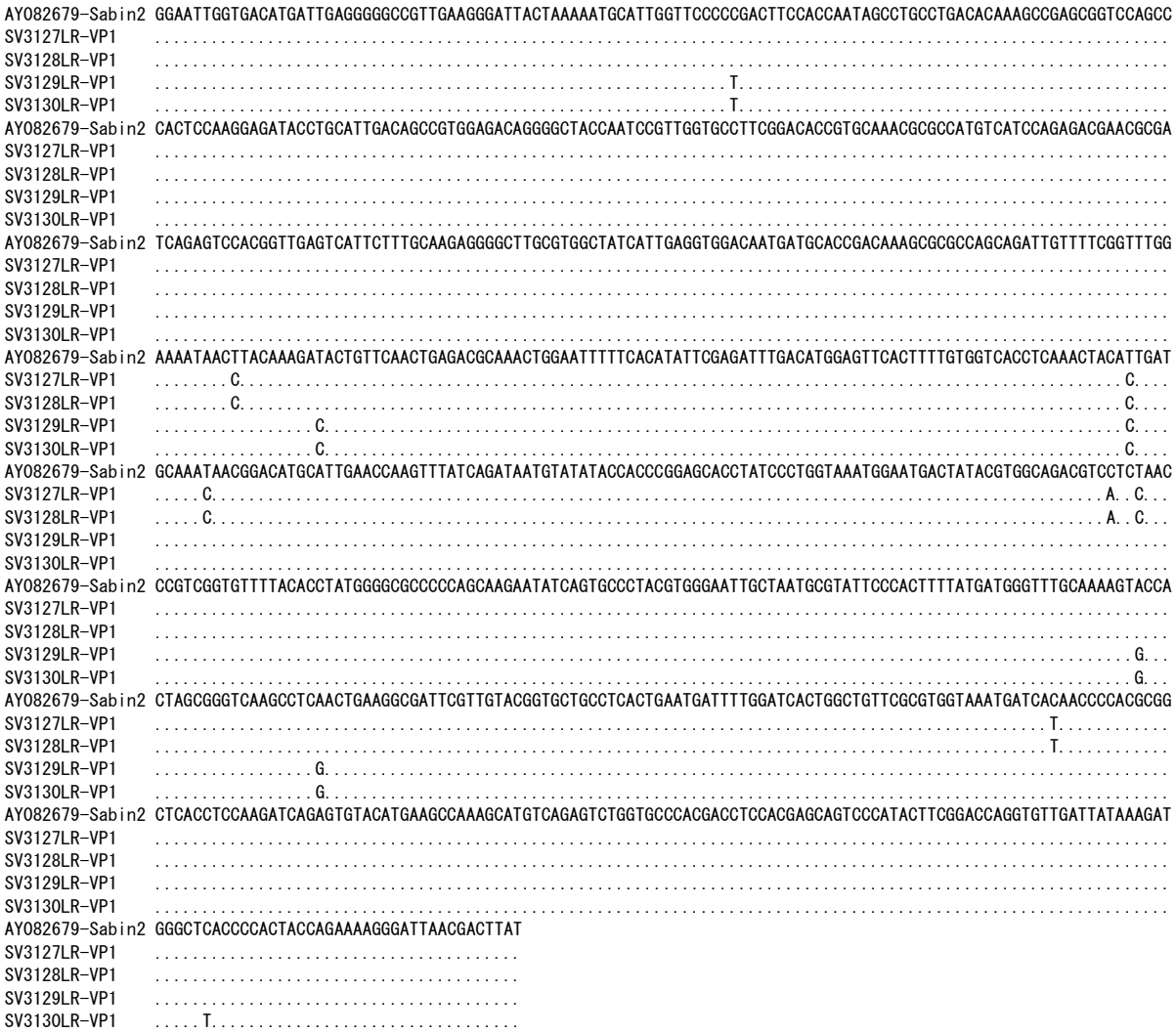

Figure 8. Nucleotide sequences of VP1 region of two VDPV2 strains.

Of particular importance is that two cases of acute flaccid paralysis reported in 2012 were caused by vaccine-derived poliovirus type 2. These cases are described below.

AFP 1 reported in Soc Trang: 19- child was not vaccinated against polio. Acute flaccid paralysis in a female was reported on 14 February 2012 in a coastal area with a humid tropical climate and poor sanitation, where more than 80% of the population are ethnic minorities. The girl was sent to hospital for treatment. Two strains of poliovirus were isolated from two stool samples collected at appropriate times and were classified as VDPV type 2 by intratypic differentiation. Poliovirus testing was

negative at 30 days after onset of paralysis. No residual paralysis was observed at 60 days. The final diagnosis was acute flaccid paralysis in an unvaccinated child.

AFP 2 in Dong Nai: A five-year-old child has received only two doses of triple-antibody oral polio vaccine, the last dose administered more than 30 days before the onset of paralysis. Acute flaccid paralysis was reported on 17 April 2012. The child lives in a mountainous area with difficult access to vaccination. The population in the area consists of 11 ethnic groups. The boy was hospitalized. Two adequate specimens collected from him yielded two strains of poliovirus, classified as VDPV type 2. No poliovirus was detected in specimens from day 30 after the onset of paralysis. No residual paralysis was detected when the boy was examined 60 days after the onset of paralysis. The final diagnosis was acute flaccid paralysis in an incompletely vaccinated child.

In 2016, Vietnam changed its immunization schedule from the three-component oral poliovirus vaccine tOPV to the two-component oral polio vaccine bOPV. In late 2018, the inactivated IPV vaccine was introduced for vaccination of infants aged five months. This has had a positive impact on the circulation of Sabin polioviruses in the population and reduced the incidence of AFP and vaccine-associated paralytic poliomyelitis (VAPP). The inactivated vaccine does not pose a risk of AFP and VAPP, but it does not reduce the risk of circulation of either vaccine-derived or wild polioviruses. Importantly, during the analyzed period, no patient with a primary diagnosis of acute flaccid paralysis had residual paralysis. Thus, no cases of AFP classified as "vaccine-associated paralytic poliomyelitis" (VAPP) have been reported in South Vietnam.

3.1.2. Isolation of non-polio enteroviruses from patients with acute flaccid paralysis in South Vietnam in 2010-2021

In addition to polioviruses, acute flaccid paralysis can also be caused by non-polio enteroviruses (NPEVs). Non-polio enteroviruses are likely to become the major pathogen after polio is eradicated. Table 5 shows the number of NPEVs isolated from AFP cases in children under 15 years of age during 2010-2021 in South Vietnam.

Table 5

Isolation of non- polio enteroviruses from AFP cases in 2010-2021

	Years												Total
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	
Number of samples	109	189	291	227	136	175	165	194	173	177	225	82	2143
Number of isolated NPEV	12	19	35	23	15	17	14	33	22	15	22	22	249
Percentage of isolated NPEV	11,1	10,1	12,0	10,1	11,0	9,7	8,5	17,0	12,7	8,5	9,8	26,8	11,8%

During twelve years of surveillance of acute flaccid paralysis, 249 non-polio enteroviruses (NPEV) of different types were isolated from patients with a primary diagnosis of AFP (2143). The percentage of NPEV isolation was 11.6%. The proportion of enteroviruses isolated from patients with AFP was almost the same throughout the study period (from 8.5% to 17%). The proportion of isolated non-polio enteroviruses was higher in 2017 (17%), when the circulation of these viruses in the country and especially in the southern provinces of Vietnam was active. This figure was the highest in 2021 (26.8%). However, this is not associated with the level of circulation of non-polio enteroviruses. The increase in the percentage of NPEV isolation was influenced by the unusually small number (82) of reported and investigated AFP cases in South Vietnam (Figure 9).

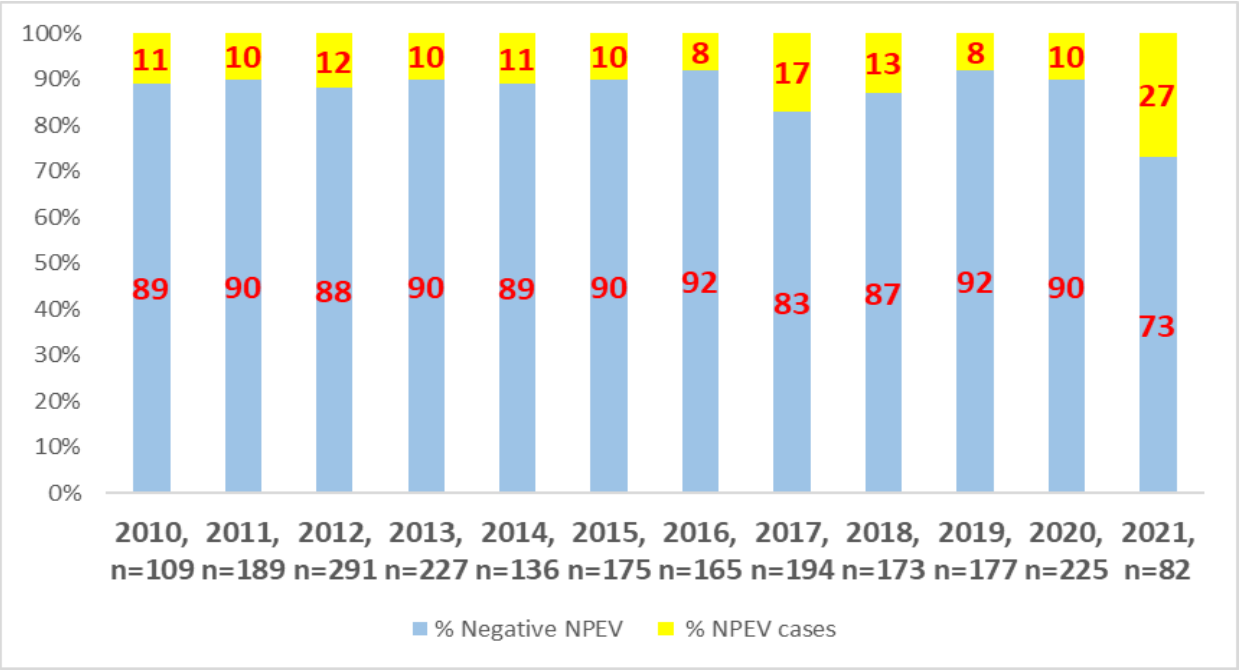

Figure 9. Proportion of non-polio enteroviruses isolated from AFP cases in 2010-2021.

The spectrum of enteroviruses isolated from patients with acute flaccid paralysis during 12 years of AFP surveillance was large. Non-polio enteroviruses of 34 types were identified in samples from cases diagnosed with AFP. Ten non-polio enteroviruses detected in samples from patients could not be typed (Table 6).

Table 6

Non-polio enterovirus types identified in patients AFP in 2012-2021

Year	Viruses	Non-polio types enteroviruses
2010	12	1CVA10, 1CVB4, 1CVB5, 1E6, 2E29, 1E30, 1EVA71, 1EVB75, 1EVB81, 1EVB84
2011	19	1CVA4, 1CVB1, 1CVB3, 2CVB5, 1E3, 1E9, 2E14, 1E25, 1E33, 3EVA71, 3EVA7 1EVB88
2012	35	1CVA9, 1CVA10, 1CVB5, 1E3, 1E11, 2E14, 1E33, 23EVA71, 3EVB79
2013	23	3CVA10, 1CVA14, 1CVA24, 2CVB2, 1CVB3, 2CVB4, 1E6, 1E7, 4E11, 1E2 5EVA71,
2014	15	1CVA6, 2CVA10, 1CVA16, 3E11, 1E30, 2EVA71
2015	17	1CVA9, 1CVA16, 2CVB2, 1E3, 1E6, 1E9, 1E11, 1E25, 7EVA71
2016	14	1CVA4, 1CVA9, 6CVA10, 1CVA21, 1CVB5, 1E11, 3E18
2017	33	13CVA16, 4CVB4, 5CVB5, 1E3, 2E7, 1E9, 2E11, 2E13, 2E14, 1EVB81
2018	22	1CVA9, 5CVA10, 1CVA16, 2CVB2, 2E6, 1E11, 2E20, 8EVA71
2019	15	2CVA10, 1CVA16, 1CVB4, 2E3, 2E11, 1E14, 5E18, 1EVA71
2020	22	10CVA4, 2CVA8, 4CVA10, 1E6, 3E14, 2EVA71
2021	22	1CVA4, 1CVB1, 3CVB3, 2E16, 15EVA71
Total	249/239	67EVA71, 4EVB75, 3EVB79, 2EVB81, 1EVB84, 1EVB88, 13CVA4, 1CVA6, 2CVA8, 4CVA9, 24CVA10, 1CVA14, 17CVA16, 1CVA21, 1CVA24, 2CVB1, 6CVB2, 5CVB3, 8CVB4, 10CVB5, 6E3, 6E6, 3E7, 3E9, 15E11, 2E13, 10E14, 2E16, 8E18, 2E20, 3E25, 2E29, 2E30, 2E33

Among the 239 NPEVs from AFP patients whose type was determined, there were viruses belonging to different HEV species. The proportion of 125 species A viruses (mostly EVA71 viruses) was the highest (52%). Species B viruses (47%) were characterized by a diversity of types. Species C viruses accounted for the smallest proportion (2.1%) and no species D viruses were isolated (Table 7 and Figures 10, 11).

Table 7

Distribution of non-polio enteroviruses by species in 2010-2021

Species of HEV	YEAR												Total
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	
HEV-A	2	4	24	9	6	8	7	13	14	4	18	16	125
HEV-B	9	14	10	12	4	8	6	20	8	11	4	6	112
HEV-C			-	1	-	-	1	-	-	-	-	-	2
Total	11	18	34	22	10	16	14	33	22	15	22	22	239

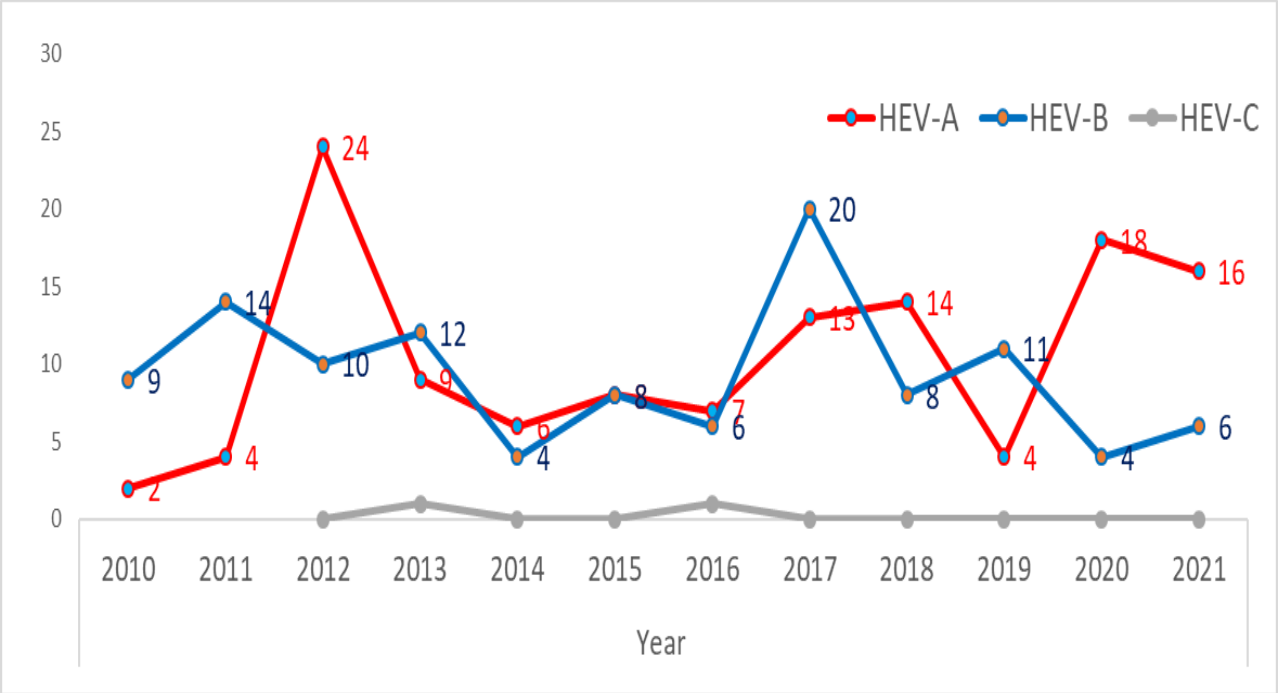

Figure 10. Non-polio enteroviruses of species A, B and C from AFP cases by years in 2010-2021

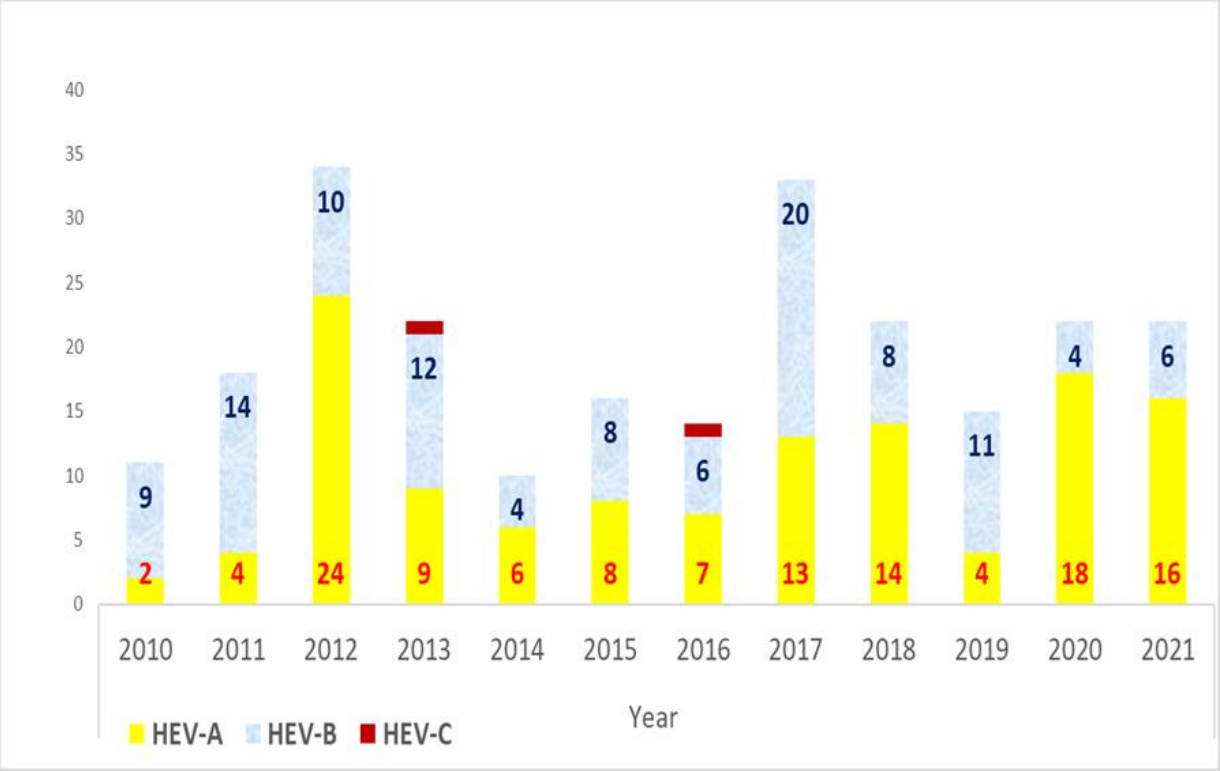

Figure 11. Distribution of non-polio enteroviruses of species A, B and C identified in patient with AFP cases by years in 2010-2021.

Among enteroviruses belonging to type A, enteroviruses 71 (67) constituted the majority; they actively circulated among the population during all years of the study, with the exception of 2016-2017, when not a single case of AFP associated with a virus of this type was registered (Table 8). In 2012, the largest number of AFP cases were associated with EVA71 (23 out of 24 AFP cases). In 2021, 15 cases were associated with EVA71, their percentage was high (94%).

Table 8

Enteroviruses of species A identified in patients with AFP in 2010-2021

Species A viruses	Years												Total
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	
EVA71	1	3	23	5	2	7	-	-	8	1	2	15	67
Coxsackievirus A4	-	1	-	-	-	-	1	-	-	-	10	1	13
Coxsackievirus A6	-		-	-	1	-	-	-	-	-	-	-	1
Coxsackievirus A8	-		-	-	-	-	-	-	-	-	2	-	2
Coxsackievirus A10	1		1	3	2	-	6	-	5	2	4	-	24
Coxsackievirus A14			-	1	-	-	-	-	-	-	-	-	1
Coxsackievirus A16			-	-	1	1	-	13	1	1	-	-	17
Total	2	4	24	9	6	8	7	13	14	4	18	16	125

Coxsackievirus A10 (24) were in second place and also showed high activity, with CVA10-related cases reported in eight of the 12 years. The number of Coxsackievirus A16 strains was also high, particularly in 2017, when they accounted for 13 of the 17 strains identified. Coxsackievirus A4 (13) were active in 2020 (10 of 13 CA4 viruses).

Table 9 shows that enteroviruses of specie B belonged to a larger number of types (25 types) than enteroviruses of type A (only seven types).

Table 9

Distribution of NPEV types of specie B isolated in 2010-2021

Types of specie B	Year												total
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	
EV75	1	3	-	-	-	-	-	-	-	-	-	-	4
EV79	-	-	3	-	-	-	-	-	-	-	-	-	3
EV81	1	-	-	-	-	-	-	1	-	-	-	-	2
EV84	1	-	-	-	-	-	-	-	-	-	-	-	1
EV88	-	1	-	-	-	-	-	-	-	-	-	-	1
Coxsackievirus A9	-	-	1	-	-	1	1	-	1	-	-	-	4
Coxsackievirus B1	-	1	-	-	-	-	-	-	-	-	-	1	2
Coxsackievirus B2	-	-	-	2	-	2	-	-	2	-	-	-	6
Coxsackievirus B3	-	1	-	1	-	-	-	-	-	-	-	3	5
Coxsackievirus B4	1	-	-	2	-	-	-	4	-	1	-	-	8
Coxsackievirus B5	1	2	1	-	-	-	1	5	-	-	-	-	10
Echovirus 3	-	1	1	-	-	1	-	1	-	2	-	-	6
Echovirus 6	1	-	-	1	-	1	-	-	2	-	1	-	6
Echovirus 7	-	-	-	1	-	-	-	2	-	-	-	-	3
Echovirus 9	-	1	-	-	-	1	-	1			-	-	3
Echovirus 11	-	-	1	4	3	1	1	2	1	2	-	-	15
Echovirus 13	-	-	-	-	-	-	-	2	-	-	-	-	2
Echovirus 14	-	2	2	-	-	-	-	2	-	1	3	-	10
Echovirus 16	-	-	-	-	-	-	-	-	-	-	-	2	2
Echovirus 18	-	-	-	-	-	-	3	-	-	5	-	-	8
Echovirus 20	-	-	-	-	-	-	-	-	2	-	-	-	2
Echovirus 25	-	1	-	1	-	1	-	-	-	-	-	-	3
Echovirus 29	2	-	-	-	-	-	-	-	-	-	-	-	2
Echovirus 30	1	-	-	-	1	-		-	-	-	-	-	2
Echovirus 33	-	1	1	-	-	-	-	-	-	-	-	-	2

Echoviruses 25, 29, 30, 33 and enteroviruses EV-75, 79, 81, 84 and 88 appeared only sporadically during the first 5 years of AFP observation and disappeared in the following years. The remaining Coxsackieviruses A8 and echoviruses belonging to enterovirus specie B were rarely detected at the beginning of the study, then their number increased from 2013 to 2018 and gradually decreased again by 2021. Echoviruses 6, 11, 14, 18 and Coxsackieviruses B2, B3, B4 and B5 accounted for the majority of identified enteroviruses of type B (60.7%).

Over the course of 12 years, enteroviruses of type C (Coxsackievirus A21 and Coxsackievirus A24) were detected only twice. The number of specie C viruses was insignificant, Coxsackievirus A24 was detected in 2013, and Coxsackievirus A21 in 2016.

Over 12 years of observation, all 2.143 children had a typical clinical picture of acute flaccid paralysis. Children under three years of age and children over three years of age were represented equally. Among those with AFP, the percentage of boys (61.4%) significantly exceeded ($p<0.05$) the percentage of girls (38.6%). Among 249 children diagnosed with AFP who were found to have enteroviruses, the majority (69.4%) were under three years of age, and 30.8% of children were over three years of age. Among them, the proportion of boys (56.2%) also exceeded the proportion of girls (43.8%).

It is important to note that 89.16% of children diagnosed with AFP, from whom non-polio enteroviruses were isolated, received three doses of OPV, and for each age group, the proportion of children vaccinated with three doses of OPV was more than 86% (Table 10).

Table 10

Information on vaccination of patients diagnosed with AFP and isolation of non-polio enteroviruses in 2010-2021

Characteristics of children by age and gender		Total number of AFP cases (%)	AFP cases with isolation of NPEV		
			Number of NPEV cases (%)	Vacinatied children of EVI cases	
				3 doses OPV	0, 1, 2 doses OPV
Age	0-12 months	317 (14.8)	44 (17.7)	38 (86.36%)	6 (13.64%)
	13-36 months	787 (36.7)	129 (51.8)	112 (86.82%)	17 (13.18%)
	3-14 years	1.039 (48.5)	76 (30.5)	72 (94.74%)	4 (5.26%)
Total		2.143	249	222 (89.16)	27 (10.84)
Gender	Boys	1.316 (61.4)	140 (56.2)	124 (88.57%)	16 (11.43%)
	Girls	827 (38.6)	109 (43.8)	98 (89.91%)	11 (10.19%)

According to the recommendations of the World Health Organization, the detection rate of acute flaccid paralysis cases should be at least 1 case per 100 000 children under 15 years of age. Two adequate stool specimens should be delivered to the laboratory no later than 14 days from the onset of paralysis in at least 80% of cases. These are the two most important indicators of surveillance quality. The AFP surveillance system in South Vietnam is working effectively, with both epidemiological and virological surveillance indicators exceeding WHO targets between 2010 and 2021.

The results showed that the AFP case detection rate was 1.63 per 100 000 children under 15 years of age (range 0.7–2.77), and the proportion of AFP cases with two adequate stool specimens delivered in a timely manner and tested in the virology laboratory reached 100%. Although the AFP surveillance system met the WHO target, it dropped to 0.7 in 2021 during the COVID-19 pandemic. The AFP case detection rate was consistently higher among boys (61.4%) than girls (38.6%). In South Vietnam, from 2010 to 2021, out of 22 polioviruses isolated, 20 were vaccine-derived strains, and two polioviruses isolated from patients who were not vaccinated or received insufficient doses of OPV were classified as vaccine-derived poliovirus type 2 (VDPV2).

The percentage of non-polio enteroviruses isolated was 11.6%. Enteroviruses of three species and 34 types were isolated and identified. Enteroviruses of specie A (mainly enterovirus 71) were represented in the largest proportion (52%). Enterovirus 71 was detected in patients with AFP more often than other types of enteroviruses. In most countries, especially in the Southeast Asian region, enterovirus 71 is also the most frequently detected type of NPEV during virological surveillance of acute flaccid paralysis.

3.2 ETIOLOGICAL CHARACTERISTIC OF VARIOUS FORMS OF ENTEROVIRUS INFECTION IN SOUTH VIETNAM

3.2.1 Epidemic situation of enterovirus infection in South Vietnam during years 2012-2021. Incidence of hand, foot and mouth disease

The main component of enterovirus infection surveillance in Vietnam is the monitoring of the incidence and virological diagnosis of hand-foot-and-mouth disease (HFMD). HFMD is clinically manifested by fever and maculopapular rash on the skin of the hands, feet and oral mucosa. According to the Law on the Prevention and Control of Infectious Diseases, this syndrome has been classified as a Category B infection in Vietnam, as a dangerous and potentially fatal infectious disease that can spread rapidly (National Assembly of Vietnam, 2007). In 2003, the first cases of this infection were reported in Ho Chi Minh City, South Vietnam, and were associated with enterovirus 71 [136]. In 2008, the Ministry of Health of Vietnam issued the first national guidelines for the prevention and control of this infection (Decision No. 1742/QD-BYT). According to the Order of the Ministry of Health No. 48 of 2010, the reporting system of infectious diseases in Vietnam was improved in 2011. In 2012, the National Guideline for the Control and Prevention of EVI was amended to take into account the new reporting system (Decision of the Ministry of Health No. 581/QD-BYT of 2012). According to the Circular of the Ministry of Health No. 54 of 2017, all cases of the disease in outpatients and inpatients must be reported under the reporting system.

HFMD is an endemic infection in Vietnam with a high incidence of 32,800 cases per 157,000 hospitalizations per year, as it is distributed throughout the country, covering the northern and central regions, highlands and southern regions (Ministry of Health of Vietnam, 2012-2021). South Vietnam is the most susceptible to the disease, with more than 60% of all cases in the country being reported there. From 2012 to 2021, HFMD consistently ranked among the top 10 most dangerous infectious diseases in Vietnam in terms of morbidity and mortality (Ministry of Health of Vietnam). From 2012 to 2021, a total of 632,651 cases of infection, including 104 deaths, were reported in 20 southern provinces of Vietnam. The

number of patients with HFMD ranged from 31,119 to 108,692 cases, while the number of deaths from this infection reached 41 cases. In 2012, the highest number of cases and deaths were recorded in the period from 2012 to 2016, when only inpatients were registered, and from 2017 to 2021 (Table 11). HFMD is reported throughout the country, but the highest burden of the disease is recorded in the southern provinces of Vietnam, both in terms of the number of cases, including severe cases, and in terms of fatality rate. Dong Thap Province, which ranks high among the 20 southern provinces of Vietnam, has recorded a significant increase in fatality rate: in 2012, 11 fatalities were recorded, and between 2014 and 2021, the number of fatalities from HFMD between zero and two percent.

One of the three southern provinces with the highest number of cases was Dong Thap Province. In 2012, a total of 6,188 cases were recorded there, with seven deaths. Dong Thap ranks second after An Giang Province. In 2021, the number of patients decreased significantly to 2,323 cases, but against this background, the number of severe diseases and deaths tended to increase. Ho Chi Minh City is also the place where the incidence rate remains one of the highest. The incidence rate was declining between 2012 and 2014. But in 2018, there was a new peak, when 39,925 cases were recorded during the outbreak. In recent years, the incidence rates have decreased again. The fatality rate in Ho Chi Minh City has also tended to decrease: in 2012, 5 deaths were recorded, in 2015 there were no deaths, and in 2021, two deaths were recorded.

Table 11

Number of cases and deaths in patients with enterovirus Hand foot and mouth disease in South Vietnam in 2012-2021

	2012		2013		2014		2015		2016		2017		2018		2019		2020		2021	
PROVINCE	HFMD	fatal	HFMD	fatal	HFMD	fatal	HFMD	fatal	HFMD	fatal	HFMD	fatal	HFMD	fatal	HFMD	fatal	HFMD	fatal	HFMD	fatal
An Giang	5497	11	1807	2	1974	1	1814	0	2318	0	3514	0	2917	1	3672	0	2396	0	1720	1
Baria-Vung Tau	7251	3	3361	1	4532	1	3010	0	1879	0	2946	0	2836	0	2228	0	1671	0	916	0
Bac Lieu	1564	1	972	1	1563	1	1339	0	1240	0	1669	0	2320	1	2076	0	1284	0	1069	0
Ben Tre	5987	1	4196	2	3994	0	2670	0	2392	0	3346	0	4291	1	2875	0	1422	0	727	0
Binh Duong	3471	0	1739	0	2343	0	1517	0	1071	0	4720	0	7444	2	5642	0	4514	1	1308	1
Binh Phuoc	1030	2	552	2	780	0	503	0	299	0	1455	0	1613	0	1183	0	827	0	272	0
Ca Mau	3674	0	1886	0	2935	0	2403	0	850	0	2141	0	3269	0	3655	0	1227	0	880	0
Can Tho	1868	2	936	1	1047	0	965	0	921	0	927	1	2376	0	2739	0	1600	0	1392	0
Dong Nai	7953	3	6020	1	5294	0	2886	0	2527	0	9174	0	12867	1	9926	0	8762	0	2947	0
Dong Thap	6188	7	3848	5	4525	1	3892	1	3603	0	6720	0	6920	1	6475	0	3052	0	2323	2
Hau Giang	1771	0	735	0	975	0	788	1	660	0	705	0	1021	0	966	0	438	0	335	1
Kien Giang	2920	0	1263	0	1860	2	1312	0	749	0	1642	0	2338	1	2440	1	937	0	785	3
Lam Dong	2903	0	1484	0	1931	0	1003	1	643	0	1049	0	1344	0	697	0	1299	0	381	0
Long An	4584	3	2957	1	3728	1	2679	0	2102	0	4513	0	5492	1	4087	0	3598	0	2104	1
Soc Trang	2310	0	1461	0	2526	0	1362	0	1297	0	1524	0	2249	1	2269	0	746	0	662	0
Tay Ninh	3514	0	1880	3	1528	0	1342	1	593	0	2158	0	2344	3	1808	0	823	0	509	0
Tien Giang	3904	1	2368	1	2525	0	1733	1	1351	0	2957	0	3285	2	3501	0	2437	0	1589	1
Tp HCM	10912	5	6596	1	7816	1	5304	0	4022	0	30825	0	39925	0	28851	0	18798	0	11433	0
Tra Vinh	1200	0	742	0	822	0	498	0	507	0	2431	0	1016	0	1918	0	609	0	402	0
Vinh Long	3296	2	3186	0	3291	0	2197	0	2145	0	2927	0	2825	1	3043	0	1116	0	1094	0
	81797	**41**	**47989**	**21**	**55989**	**8**	**39217**	**5**	**31169**	**0**	**87343**	**1**	**108692**	**16**	**90051**	**1**	**57556**	**1**	**32848**	**10**

In 2012–2021, the incidence of HFMD among children under 15 years of age ranged from 363 to 1239 cases per 100,000 children (Table 12 and Figure 12).

Table 12

Prevalence of HFMD in 20 provinces of South Vietnam in 2012-2021

YEAR	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
An Giang	1052	345	377	373	483	740	622	792	518	371
Baria-Vung Tau	2889	1322	1760	1122	692	1076	1025	796	589	321
Bac Lieu	740	458	733	621	572	765	1057	941	578	479
Ben Tre	1957	1370	1302	861	770	1074	1373	918	453	231
Binh Duong	825	397	515	302	206	872	1306	945	720	207
Binh Phuoc	466	247	344	219	128	616	674	488	337	109
Ca Mau	1247	639	993	824	292	736	1125	1259	423	300
Can Tho	631	314	348	329	312	312	796	912	531	459
Dong Nai	1209	893	767	411	352	1256	1733	1312	1135	383
Dong Thap	1520	943	1108	985	916	1715	1773	1667	785	597
Hau Giang	954	395	522	437	367	393	572	543	247	189
Kien Giang	697	300	439	317	180	394	560	583	223	184
Lam Dong	970	490	631	327	208	337	428	221	408	119
Long An	1292	828	1038	678	526	1117	1346	992	864	502
Soc Trang	730	461	795	453	435	515	766	778	257	226
Tay Ninh	1327	705	569	489	214	771	831	635	287	177
Tien Giang	949	572	605	413	320	695	768	816	566	367
Tp HCM	586	347	403	263	195	1467	1858	1314	838	513
Tra Vinh	485	298	329	204	207	992	414	782	248	162
Vinh Long	1311	1263	1300	884	863	1178	1137	1225	449	438
South VietNam	**998**	**578**	**667**	**461**	**363**	**1007**	**1239**	**1015**	**641**	**365**

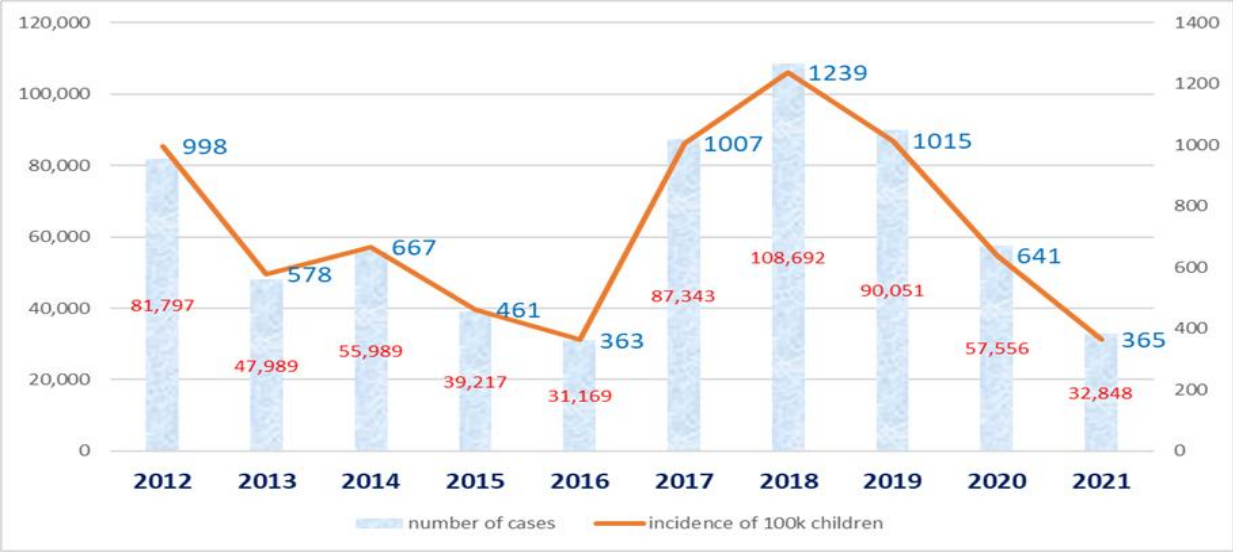

Figure 12. Number of cases and incidence rates per 100,000 children under 15 years of age in 20 provinces of South Vietnam in 2012–2021.

According to the surveillance data of HFMD, Ba Ria Vung Tau Province was the hotspot for the incidence from 2012 to 2016. Among the 20 provinces, Dong Thap had the highest number of cases since 2016. In 2018, Ho Chi Minh City, Binh Duong, Dong Nai and Long An had high incidence. All these provinces have numerous industrial zones and very high population density (Figure 13 and 14). This indicated that the etiologic agents of HFMD were circulating throughout southern Vietnam.

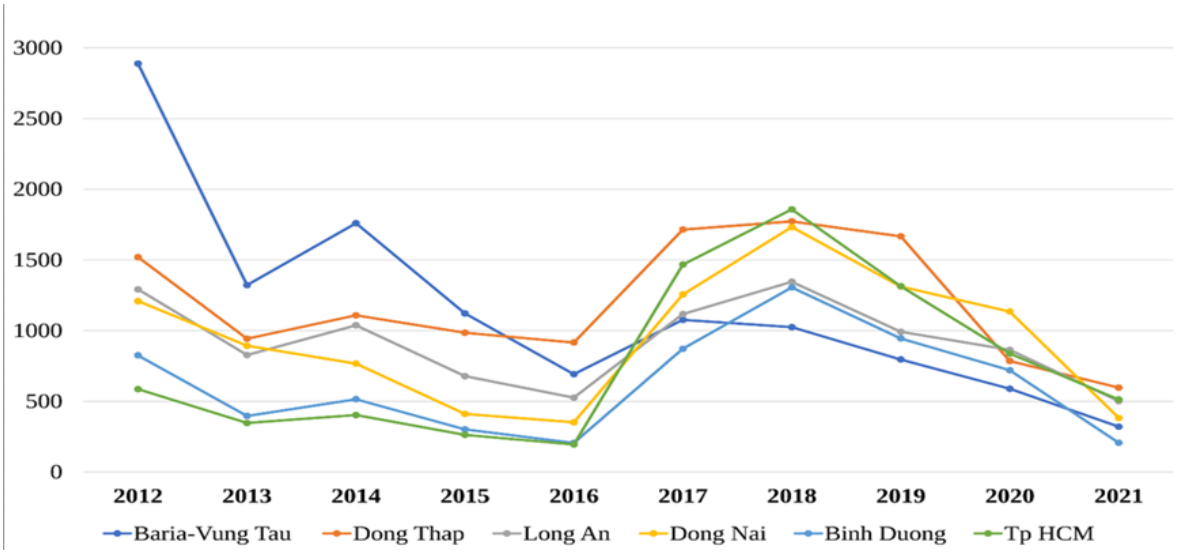

Figure 13. Dynamics of enterovirus exanthema cases in children under 15 years of age in several provinces of southern Vietnam in 2012-2021.

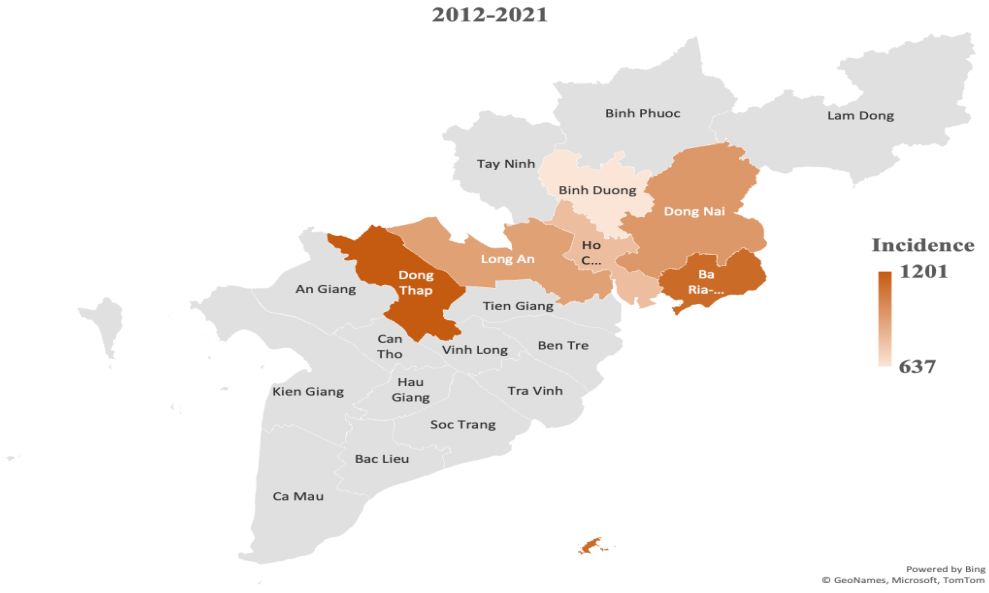

Figure 14. Six provinces in southern Vietnam with the highest number of HFMD cases among children under 15 years of age in 2012-2021.

Over a 10-year period, in six hot spots in southern Vietnam – Ba Ria, Vung Tau, Dong Thap, Ho Chi Minh City, Binh Duong, Dong Nai and Long An province with high incidence – the percentage of HFMD cases each year ranged from 49% to 70% of the total HFMD cases in southern Vietnam (Figure 15).

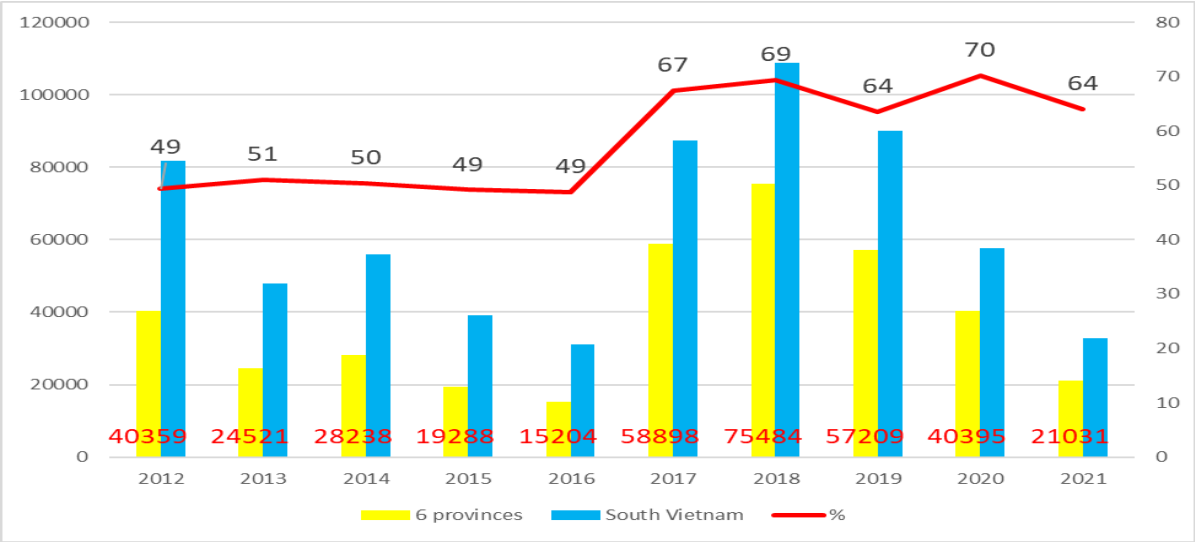

Figure 15. Percentage of HFMD cases in six provinces from the number of HFMD cases in South Vietnam in 2012-2021.

Thus, in 2012, the two provinces of Ba Ria-Vung Tau and Ben Tre had the highest number of cases among children under 15 years of age. The number gradually decreased between 2012 and 2016 before increasing again during the HFMD outbreak in 2018, and has shown a gradual downward trend in recent years. In 2021, these provinces reported 321 and 231 cases, respectively. Dong Thap province had the third highest number of cases in 2012 (1,520 cases), behind Ba Ria, Vung Tau and Ben Tre, which showed the same epidemic trend as the other two provinces. In recent years (up to 2021), the number of HFMD cases among children under 15 years of age remained high in all 20 provinces.

3.2.2 Etiological agents of hand foot mouth disease in South Vietnam

From 2012 to 2021, 7976 stool or throat swab samples were collected from children with primary diagnosis of hand-foot-and-mouth disease in South Vietnam. The children had either complicated or uncomplicated disease. Some of the children were admitted to hospitals in southern Vietnam under the HFMD sentinel

surveillance program. Virological testing detected 3700 non-polio enteroviruses, of which 3611 were identified. These enteroviruses belonged to 15 different types (Table 13).

Table 13

Types of enteroviruses in patients with HFMD in the South Vietnam in 2012-2021

Years	Number	Enteroviruses
2012	243	212EVA71, 10CVA6, 3CVA10, 15CVA16, 3NPEV
2013	191	174EVA71, 2CVA6, 3CVA10, 6CVA16, 1CVA24, 5NPEV
2014	137	90EVA71, 1CVA2, 1CVA5, 12CVA6, 19CVA10, 10CVA16, 1CVB3, 1ECHO16, 2NPEV
2015	252	157EVA71, 4CVA2, 4CVA4, 3CVA5, 37CVA6, 3CVA8, 2CVA9, 29CVA16, 1CVB4, 2E16, 10NPEV
2016	423	320EVA71, 2CVA2, 1CVA4, 3CVA5, 2CVA6, 7CVA8, 69CVA10, 9CVA16, 1CVB3, 1E18, 8NPEV
2017	578	250EVA71, 6CVA2, 9CVA4, 11CVA5, 154CVA6, 13CVA8, 6CVA10, 106CVA16, 1CVB3, 5CVB4, 17NPEV
2018	550	196EVA71, 31CVA2, 18CVA4, 17CVA5, 105CVA6, 8CVA8, 154CVA10, 4CVA16, 17NPEV
2019	560	80EVA71, 39CVA2, 15CVA4, 15CVA5, 247CVA6, 16CVA8, 53CVA10, 75CVA16, 3CVB4, 2E18, 15NPEV
2020	419	39EVA71, 4CVA2, 35CVA4, 235CVA6, 39CVA8, 35CVA10, 1CVA16, 2CVB3, 28CVB4, 1NPEV
2021	347	253EVA71, 3CVA2, 5CVA4, 11CVA5, 26CVA6, 4CVA8, 2CVA9, 17CVA10, 4CVB3, 4CVB4, 7E16, 11NPEV
Total	3700	1771EVA71, 90CVA2, 87CVA4, 61CVA5, 830CVA6, 90CVA8, 4CVA9, 359CVA10, 255CVA16, 1CVA24, 9CVB3, 36CVB4, 5CVB5, 10ECHO16, 3ECHO18, 89NPEV

Among the identified viruses, 1.8% of enteroviruses (67 strains) were classified as type B, only one virus CVA24 belonged to type C. The majority of the identified viruses, 3,543 or 98.2% of strains were classified as enteroviruses of type A (Table 14 and Figure 16).

Table 14

Enteroviruses of types A, B and C in patients with HFMD in South Vietnam in 2012-2021

Species of HEV	Year										Total
	2012	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	
HEV-A	240	185	133	237	413	555	533	540	388	319	3543
HEV-B			2	5	2	6		5	30	17	67
HEV-C		1									1
Total	240	186	135	242	415	561	533	545	418	336	3611

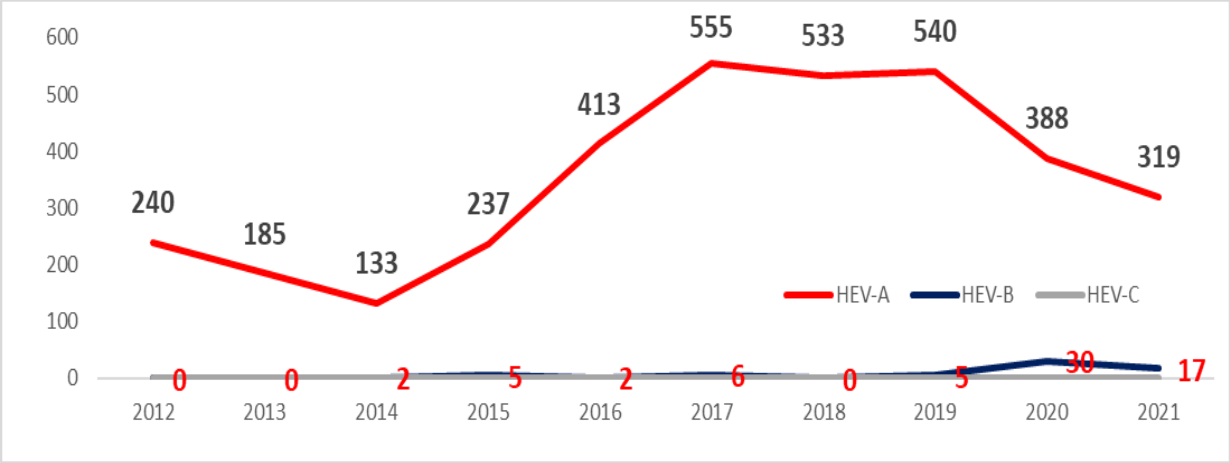

Figure 16. Detection of enteroviruses species A, B and C in patients in 2012-2021.

The figure shows the dynamics of detection of non-polio enteroviruses of different species in patients with HFMD over 12 years. The number of isolated viruses increased in 2016 and was high from 2017 to 2019, then this number began to decline.

Half of the identified enteroviruses of specie A (1771) were represented by EVA71 viruses, and another half (1772) of enteroviruses of specie A were represented by Coxsackievirus A (CVA) viruses. Among them there were enteroviruses of seven types with the prevalence of viruses of three types CAV6, CAV10 and CAV16, their percentage was 81,5% of all isolated CVA viruses. CVA6 accounted for 830 strains or 47%, CVA10 – 359 strains or 20%, CVA16 – 255 strains or 14% (Figure 17).

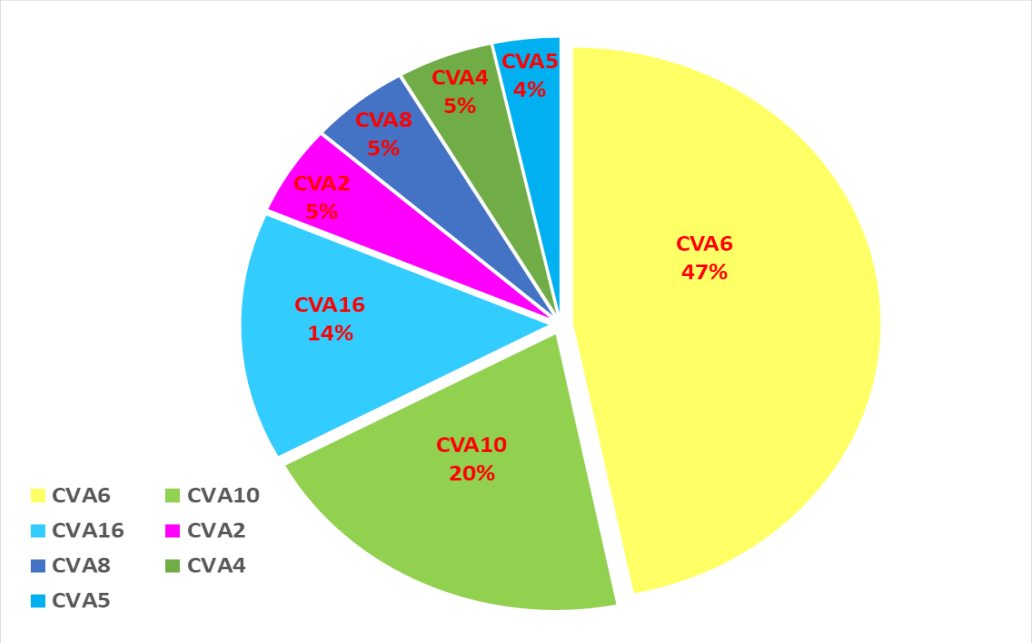

Figure 17. Proportion of enteroviruses of specie A (excluding EV71) in patients with HFMD in South Vietnam in 2012-2021.

Enteroviruses of specie B were absent in 2012, 2013 and 2018. From 2014 to 2017 and in 2019 they appeared sporadically (from two to six detected viruses per year). However, in 2020, the number of detected enteroviruses of specie B reached 30 strains (two CVB3 and 28 CVB4 viruses) and in 2021, the number of identified HEVB viruses was 17 (Figure 18).

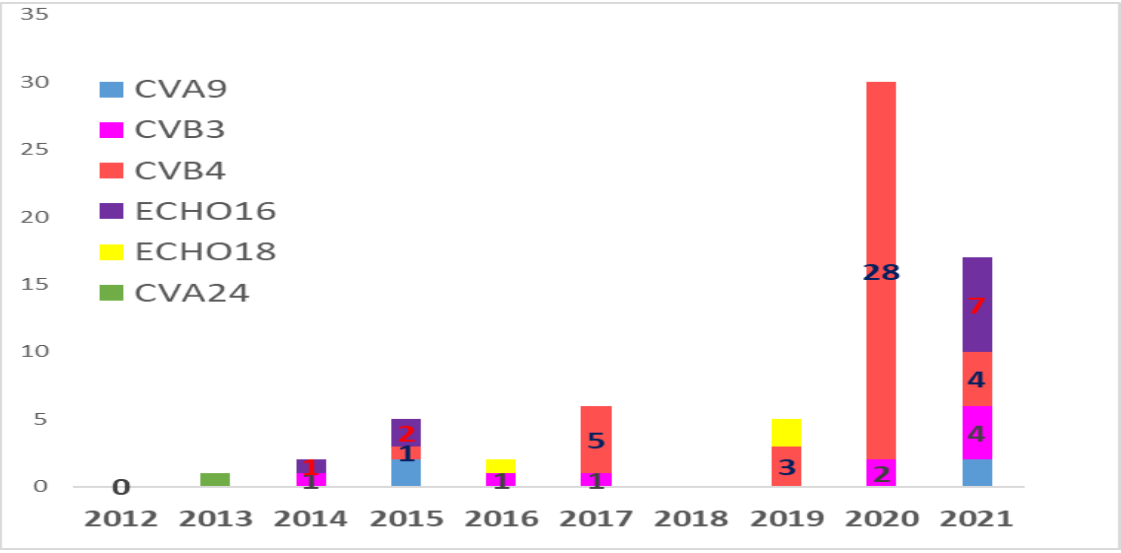

Figure 18. Detection of enteroviruses specie B in patients with HFMD in South Vietnam in 2012-2021.

The proportion of etiologic agents - enterovirus 71, CVA viruses, CVB viruses and Echoviruses changed over 10 years (Figure 14). Enterovirus 71, which was the leader in 2012-2016, gave way in 2017 to other enteroviruses of specie A, mainly Coxsackie A6, Coxsackie A10 and Coxsackie A16 viruses, which dominated from 2017 to 2020. In 2021, enterovirus 71 regained its leading position. (Figure 19).

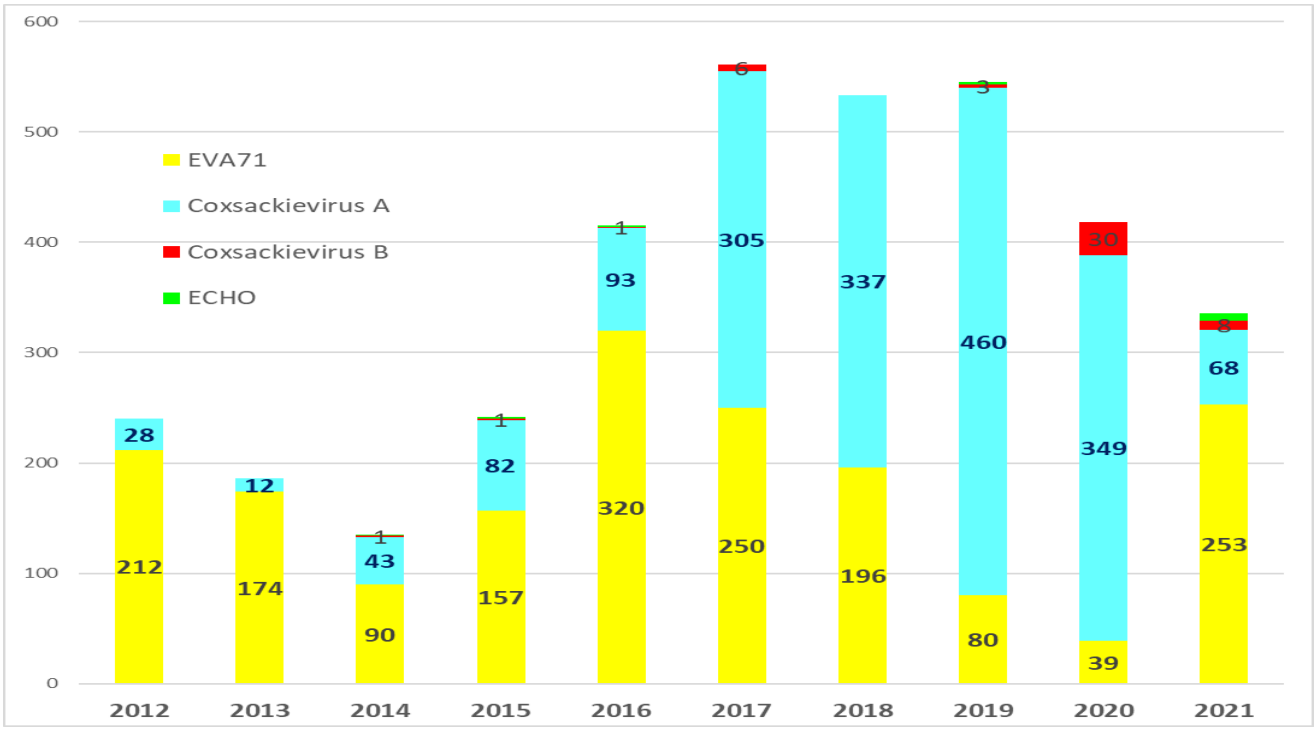

Figure 19. Dynamics of detection of specie A viruses in patients with HFMD in South Vietnam in 2012-2021.

An analysis of HFMD cases by severity showed a high percentage of complicated forms of infection with damage to various organs and systems. The proportion of clinically severe diseases (89.3%) significantly exceeded ($p<0.001$) the percentage (10.7%) of clinically mild cases of the disease with papulovesicular rash on the hands, feet, and oral mucosa, accompanied by normal or subfebrile temperature. In cases of complicated HFMD, in addition to the typical rash, patients had a high fever and numerous neurological or cardiovascular manifestations, such as muscle cramps, confusion, meningitis, or myocarditis. Some patients with the maximum severity of the disease had serious complications from the nervous, cardiovascular, and respiratory systems. Among patients with complicated HFMD of this form of infection, 33 (0.9%) fatal cases were registered. The proportion of

children under three years of age was 92.8% of the total number of children diagnosed with HFMD of the oral cavity and extremities, significantly exceeding ($p<0.001$) the proportion of children aged three to fourteen years (7.2%). Similarly, the percentage of sick boys (61.3%) significantly exceeded ($p<0.05$) the proportion for girls – 38.7% (Table 15).

Table 15

Data of patients with HFMD according clinical picture, gender and age in 2012-2021

Year	Number of patients	**Severity**			**Gender**		**Age**		
		Mild clinical form	Complicated clinical form	Fatal cases	Female	Male	0-12 months	13-36 months	3-14 years
2012	243	12	211	20	82	161	84	146	13
2013	191	2	181	8	78	113	45	135	11
2014	137	24	113	0	62	75	18	96	23
2015	252	36	216	0	106	146	37	185	30
2016	423	55	368	0	148	275	123	262	38
2017	578	51	527	0	221	357	119	438	21
2018	550	32	516	2	218	332	113	414	23
2019	560	127	433	0	219	341	129	364	67
2020	419	37	382	0	167	252	100	308	11
2021	347	18	326	3	130	217	63	256	28
Total	3700	394 (10.7%)	3273 (88.4%)	33 (0,9%)	1431 (38.7%)	2269 (61.3%)	831 (22.4%)	2604 (70.4%)	265 (7.2%)

Hand, foot and mouth disease is the most common clinical form of enterovirus infection in South Vietnam. In Vietnam, many changes have been made in the guidelines for diagnosis, treatment, monitoring and prevention of hand and mouth disease over the years, but the incidence trend has continued to increase in recent years. Hand, foot and mouth disease is a serious public health problem in Vietnam due to the active circulation of enteroviruses. Due to the high morbidity and severe complications of HFMD, which is the most common infection in Southeast Asia, the economic and social burden of enterovirus infection is very high for South Vietnam as well as for the whole country.

3.2.3 Etiological agents of enterovirus infection in the form of enterovirus meningitis or meningoencephalitis

According to the surveillance system of Vietnam, the National Laboratory for Polio and Enterovirus Infection Diagnosis in South Vietnam has been continuously and effectively testing biological materials from children with severe clinical forms such as enterovirus meningitis and encephalitis.

From 2012 to 2021, stool or throat swab samples from hospitalized children diagnosed with enteroviral meningitis or encephalitis from 20 provinces in the southern provinces of Vietnam were collected and tested using virological methods. Among the 228 strains detected, enteroviruses of 24 types were identified. Among these viruses, representatives of two specie of enteroviruses A and B predominated, with enteroviruses of specie A accounting for 57% and enteroviruses of specie B accounting for 42.5% of the detected viruses. In 2018, only one enterovirus D68 (0.5%), belonging to specie D of enteroviruses, was also detected (Tables 16 and 17)

Table 16

Etiological agents of meningitis and meningoencephalitis cases in 2012-2021

Year	Number	Enterovirus types
2012	30	18EVA71, 2CVA12, 2CVB2, 1CVB3, 4CVB4, 1E9, 2NPEV
2013	20	15EVA71, 2CVA12, 1CVB1, 1CVB3, 1NPEV
2014	26	16EVA71, 1CVA9, 1CVA12, 4CVB3, 1CVB5, 1E9, 1E11, 1NPEV
2015	40	10EVA71, 1CVA2, 1CVA8, 1CVA9, 3CVB3, 8CVB4, 3CVB5, 2E6, 2E9, 3E11, 1E25, 3E30, 2NPEV
2016	24	15EVA71, 1CVA2, 2CVB2, 2CVB3, 1CVB4, 1CVB5, 2NPEV
2017	22	3EVA71, 1CVA5, 3CVA6, 1CVA8, 2CVA16, 5CVB4, 1CVB5, 3E5, 1E9, 1E21, 1NPEV
2018	28	6EVA71, 1EVD68,3CVA2, 3CVA6, 2CVB1, 1CVB2, 3CVB5, 2E6, 1E9, 4E11, 2NPEV
2019	28	4EVA71, 1CVA2, 2CVA6, 1CVA16, 1CVB1, 2CVB5, 1E3, 3E9, 7E11, 4E25, 2NPEV
2020	11	4EVA71, 2CVA6, 1CVA8, 1CVB3, 1E30, 2NPEV
2021	15	5EVA71, 2CVA4, 3CVA6, 1CVA8, 1CVB1, 1CVB4, 1E21, 1NPEV
Всего	244/ 228	96EVA71, 6CVA2, 2CVA4, 1CVA5, 13CVA6, 4CVA8, 5CVA12, 3CVA16, 2CVA9, 5CVB1, 5CVB2, 12CVB3, 19CVB4, 11CVB5, 1E3, 3E5, 4E6, 9E9, 15E11, 2E21, 5E25, 4E30, 1EVD68,16NPEV

Table 17

Strains of different HEV species isolated from patients with meningitis or meningoencephalitis

Species of HEV	Year										Total
	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	
HEV-A	20	17	17	12	16	10	12	8	7	11	130 (57%)
HEV-B	8	2	8	26	6	11	13	18	2	3	97 (42,5%)
HEV-C	-	-	-	-	-	-	1	-	-	-	1 (0,5%)
Total	28	19	25	38	22	21	26	26	9	14	228

During the study, the frequency of detection of enteroviruses of species A and B from patients diagnosed with meningitis or meningoencephalitis was not the same in different years. In 2012-2014, 2016 and 2021, there was a clear predominance of enteroviruses of specie A among isolated and identified viruses, and in 2015 and 2019, enteroviruses of specie B predominated (Figures 20 and 21).

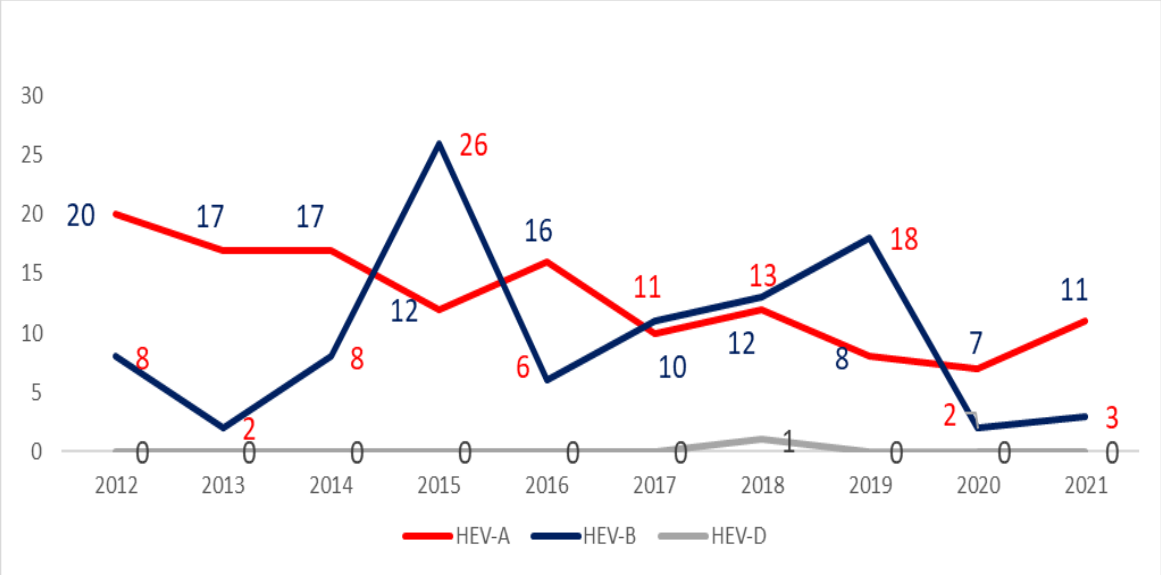

Figure 20. Number of viruses belonged to different HEV species isolated in patients with meningitis and meningoencephalitis in 2012-2021.

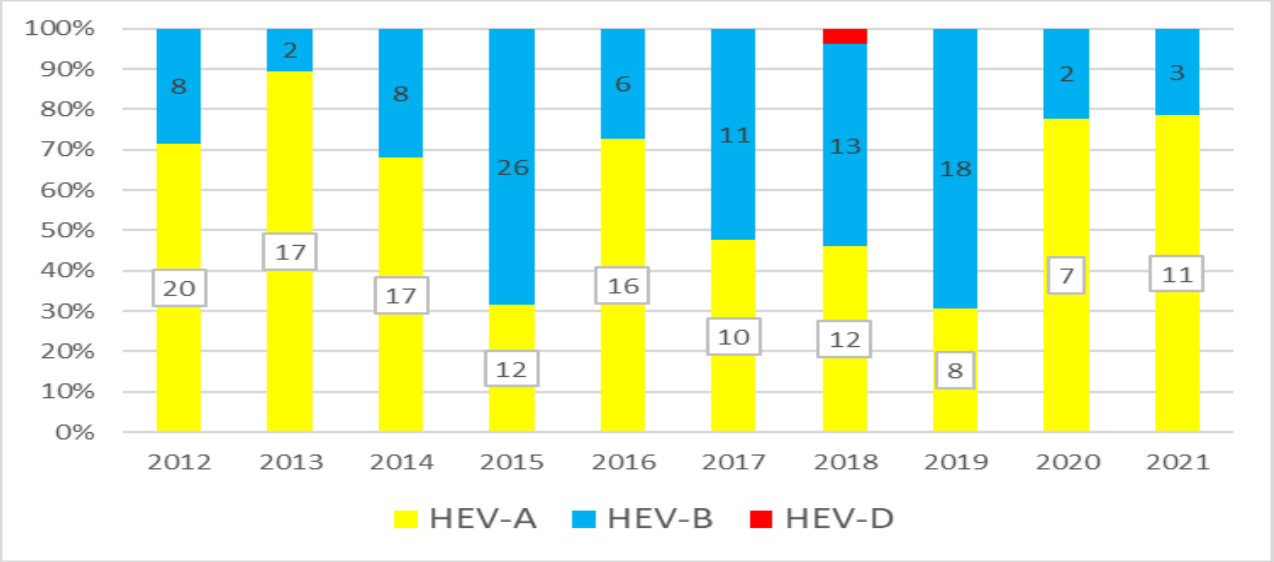

Figure 21. Frequency of detection of enteroviruses belonged to different HEV species in patients with meningitis and meningoencephalitis in 2012-2021.

The higher proportion of enteroviruses of specie A in patients with this clinical form of EVI is associated with the unusually wide circulation of enterovirus 71 in South Vietnam, which was the clear leader among all viruses of type A, 96 strains of this type of virus made up 74%. Among viruses of type A, the second place was taken by CVA6 (10% of 130 viruses of specie A). The proportion of the remaining Coxsackievirus A of six types (CVA2, 4, 5, 8, 12 and 16) was equal to 16% of the total number of enteroviruses of specie A. All of them, like enterovirus 71, are typical causative agents of enterovirus infection in the HFMD form (Figure 22).

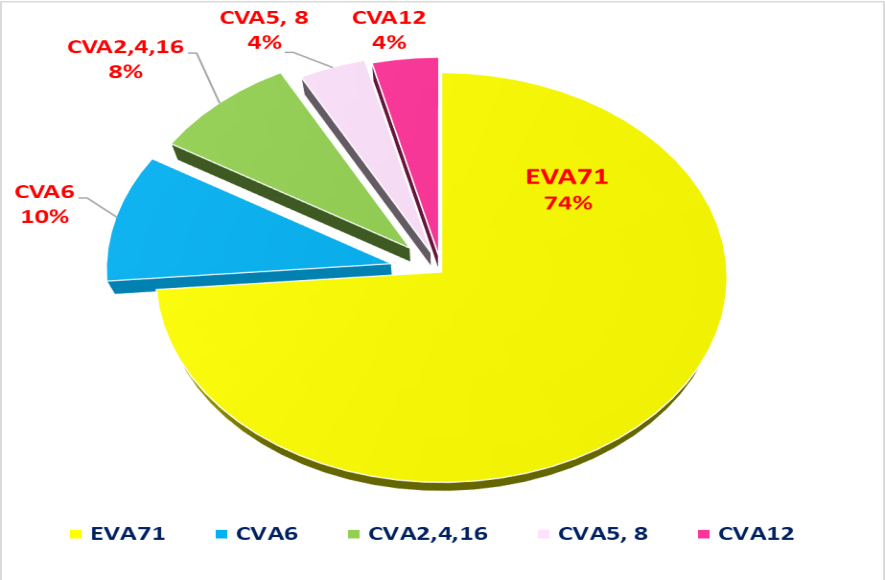

Figure 22. Types of enteroviruses of species A isolated from patients with meningitis or encephalitis in South Vietnam in 2012-2021.

For enteroviruses belonging to specie B, diversity (14 types) is greater than for viruses of type A (Figure 23). Echoviruses accounted for 42.5% of enteroviruses detected in patients with enteroviral meningitis or meningoencephalitis. Their share was higher than that of type A enteroviruses (excluding enterovirus 71). Among specie B enteroviruses, the share of Coxsackie viruses B1, B2, B3, B4, and B5 was the largest – 53.6% (52 viruses). Among Coxsackievirus B viruses, the largest share is accounted for by Coxsackievirus B4 (20%), followed by Coxsackie virus B3 (12%), Coxsackievirus B5 (11%) and Coxsackievirus B1 and B2 viruses, which together accounted for 10%. The share of Echoviruses was 44.4% (43 viruses) and among them the leader was Echovirus 11 (15 viruses). Most of the Coxsackievirus B viruses, as well as Echovirus 11, along with Echovirus 6, Echovirus 9 and Echovirus 30 viruses are known as typical etiologic agents of enteroviral meningitis and meningoencephalitis.

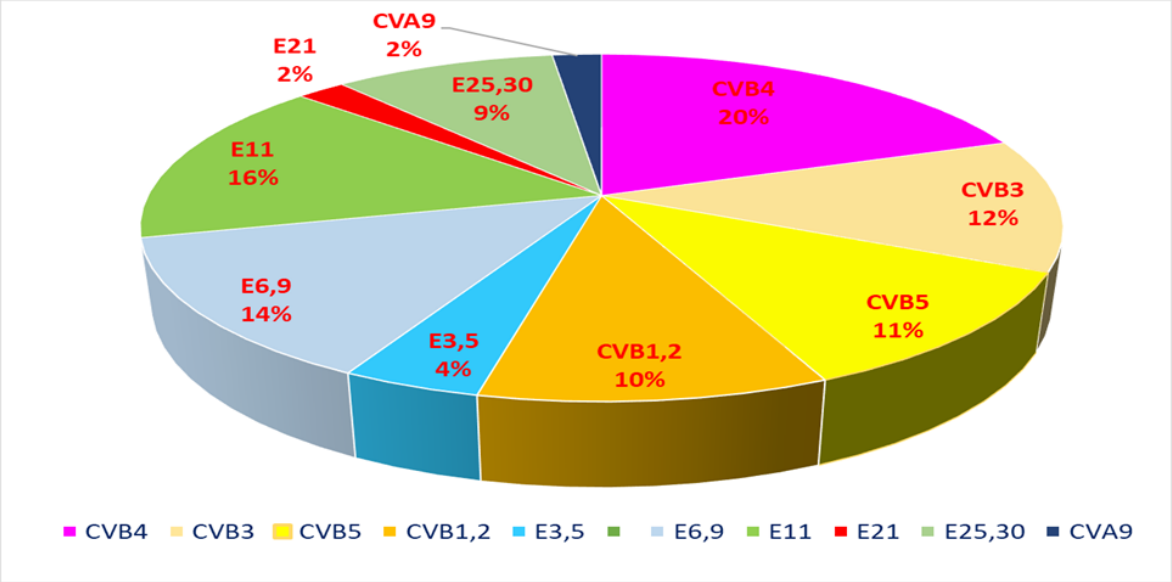

Figure 23. Types of enteroviruses of specie B isolated from patients with meningitis or meningoencephalitis in South Vietnam in 2012-2021.

All the enteroviruses (21 types) detected in patients diagnosed with meningitis and meningoencephalitis (excluding Coxsackie virus A9, which belongs to specie B) can be divided into four conditional groups (Table 18). Enterovirus 71 – 42.7% (96 of 225 strains) should be considered separately due to the incomparably wide and active circulation of EV 71 in Southeast Asia. And three more groups: Coxsackievirus A viruses – 15.1% (34 of 225 strains), Coxsackievirus B viruses – 23.1% (52 of 225 strains) and Echoviruses – 19.1% (43 of 225 strains).

Table 18

Enteroviruses isolated from patients with meningitis or meningoencephalitis in South Vietnam in 2012-2021

Group of EV	Year										Total
	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	
EVA71 (1)	18	15	16	10	15	3	6	4	4	5	96
Coxsackievirus A (2)	2	2	1	2	1	7	6	4	3	6	34
Coxsackievirus B (3)	7	2	5	14	6	6	6	3	1	2	52
Echoviruses (4)	1	-	2	11		5	7	15	1	1	43
Total	28	19	24	37	22	21	25	26	9	14	225

The importance of these groups, as well as their shares, varied over the years (Figure 24). Enterovirus 71 dominated in 2012-2014 and 2016 (64-79%, and 68%).

Coxsackievirus B viruses combined with Echoviruses were in the lead in 2015 (68%) and 2019 (69%), their share was also high in 2017 (52.4%) and 2018 (52%). These data reflect the role of Coxsackievirus B1-5 viruses and Echoviruses as typical etiologic agents of enteroviral meningitis/meningoencephalitis.

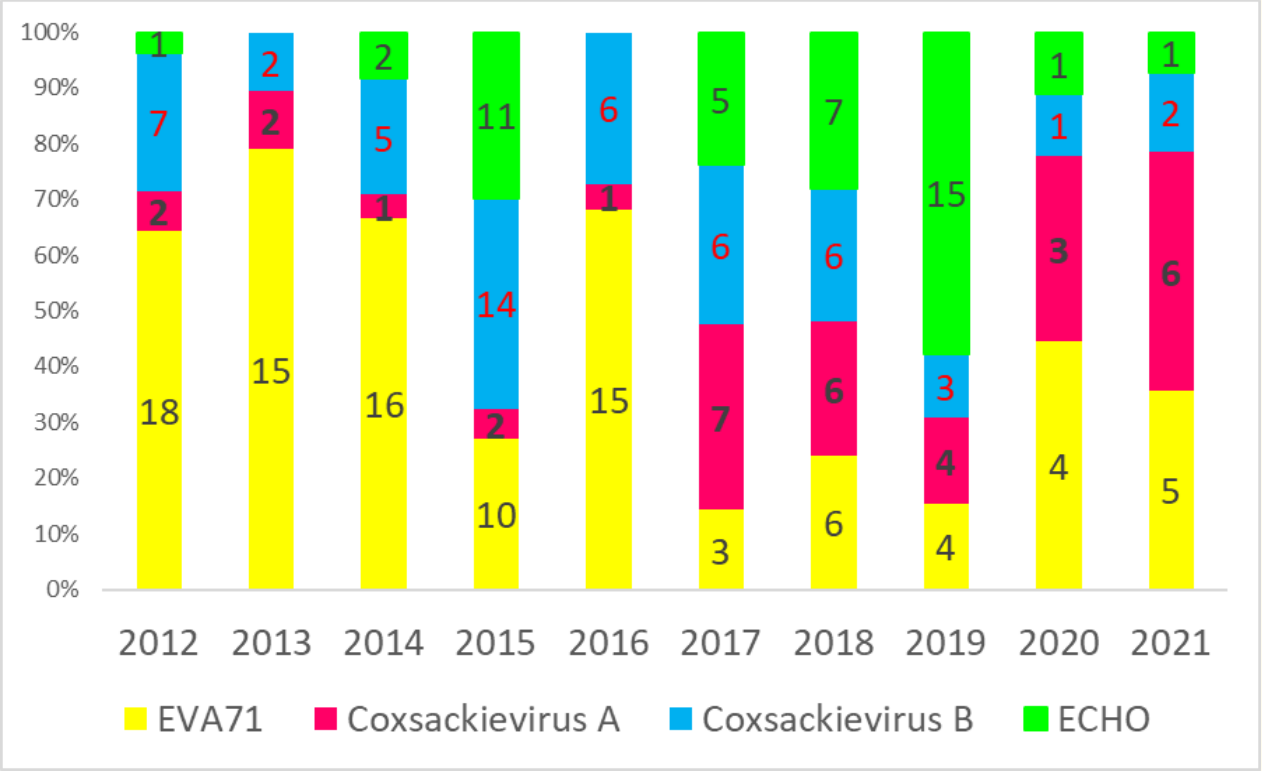

Figure 24. Proportion of 4 different groups of enteroviruses isolated from patients with meningitis/meningoencephalitis in South Vietnam in 2012-2021.

Most children with enteroviral meningitis or meningoencephalitis (92.2%) were under three years of age, and only 7.8% of children were over three years of age. Among all 244 children, the proportion of boys (68%) significantly exceeded ($p<0.05$) the proportion of girls (32%). The percentage of fatal outcomes was 12.7% (Table 19).

Table 19

Characteristic of children with enteroviral meningitis or meningoencephalitis by gender and age

Year	Meningitis or meningoencephalitis	Fatal cases (%)	**Gender**		**Age**		
			Female	Male	0-12 months	13-36 months	3-14 years
2012	30	9 (30%)	12	18	12	18	0
2013	20	6 (30%)	3	17	6	12	2
2014	26	3 (12%)	9	17	5	20	1
2015	40	6 (15%)	14	26	11	27	2
2016	24	0	6	18	0	23	1
2017	22	1 (5%)	4	18	12	7	3
2018	28	4 (14%)	12	16	7	18	3
2019	28	0	8	20	4	19	5
2020	11	1 (9%)	4	7	3	6	2
2021	15	1 (7%)	6	9	4	11	0
Total	**244**	**31 (12.7%)**	**78 (32%)**	**166 (68%)**	**64 (26.2%)**	**161 (66%)**	**19 (7.8%)**

Thus during 12 years of this research with the help of different methods, the virologists detected non-polio enteroviruses belonged to four species of enteroviruses. Species A of enteroviruses were represented by enteroviruses of ten enterovirus types: CVA2, CVA4, CVA5, CVA6, CVA8, CVA10, CVA12, CVA14, CVA16 and EVA71, which was the leading etiological agent of various forms of enterovirus infection in South Vietnam especially of HFMD. Enterovirus EV71 together with CVA enteroviruses play very important role in the spread of infectious diseases. The number of Species B of enteroviruses was also high – CVA9, CVB1, CVB2, CVB3, CVB4, CVB5, ECHO 3, 4, 6, 7, 9, 11, 13, 18, 19, 25, 27 and 30. The role of species B viruses as etiological agents was much more important as etiological agents of enterovirus meningitis or encephalitis but not of HFMD. The detection of Species C of enteroviruses was rare – CVA21, CVA24, EV-C96 and unique detected enterovirus of species D belonged to type EVD68. Among different clinical forms of enterovirus infection in South Vietnam HFMD with its main etiological agent enterovirus 71 dominated.

3.3 THE ROLE OF ENTEROVIRUS 71 AS ETIOLOGICAL AGENT OF INFECTIOUS DISEASES IN SOUTH VIETNAM

Enterovirus 71 is mainly associated with manifestations of hand, foot, and mouth disease (HFMD). However, EV71 infections can lead to serious or even fatal neurological and cardiopulmonary complications, and it can be associated with acute flaccid paralysis (myelitis), aseptic meningitis, pulmonary oedema, and myocarditis.

Over the past 25 years, cyclical outbreaks associated with enterovirus 71 have occurred in the Asia-Pacific region, most notably outbreaks of enterovirus HFMD in Malaysia (1997), Taiwan (1998), China (2008), and Vietnam (2011). These outbreaks have resulted in hundreds or even thousands of deaths, mostly among infants and young children.

In 2003, the first case associated with enterovirus EVA71 was reported in Ho Chi Minh City, a southern province of Vietnam. The first outbreak of hand, foot, and mouth disease was reported in 2005, with Coxsackie A16 and Enterovirus 71 being the main pathogens, but only cases associated with EVA71 were associated with neurological complications [136]. Since then, an increasing number of severe cases of hand, foot, and mouth exanthema have been reported. In 2008, the disease became a notifiable disease, and at least 10,000 cases were reported annually between 2008 and 2010. A sharp increase in hospitalizations and deaths was observed in 2011–2012, with over 200,000 children hospitalized and over 200 deaths [67].

To assess the role of enterovirus EVA71 as an etiologic agent of various infectious diseases in South Vietnam, it is important to consider the following data. The number of EVA71 virus strains isolated from 2,143 examined patients diagnosed with acute flaccid paralysis was 67 strains (3.1%). From samples from 228 patients with enteroviral meningitis and/or meningoencephalitis, 96 EVA71 viruses were isolated, accounting for 42.1%. EVA71 had the closest association with cases of hand, foot, and mouth disease, where the proportion of EVA71 detected was highest 49% of identified enteroviruses. In addition, another 1,772 viruses also belonged to species A, like enterovirus 71 (Table 20). It is important to note that the percentage of

EVA71 virus strains among identified enteroviruses of type A was high in patients with all studied forms of infection.

Table 20

Isolation of EVA71 strains from patients with various clinical forms of enterovirus infection in South Vietnam

YEAR	AFP (n= 239 cases)		Meningitis/encephalitis (n= 228 cases)		HFMD (n= 3611 cases)	
	EV71	Evs non EV71	EV71	Evs non EV71	EV71	Evs non EV71
2010	1	10	No data in this research			
2011	3	15				
2012	23	11	18	10	212	28
2013	5	17	15	4	174	12
2014	2	8	16	9	90	45
2015	7	9	10	28	157	86
2016	0	14	15	7	320	95
2017	0	33	3	18	250	312
2018	8	14	6	20	196	337
2019	1	14	4	22	80	465
2020	2	20	4	5	39	379
2021	15	7	5	9	253	84
TOTAL	**67 (28%)**	**172 (72%)**	**96 (42%)**	**132 (58%)**	**1771 (49%)**	**1840 (51%)**

Over a 10-year period, there were three peaks of intense circulation of Enterovirus 71 strains in 2012, 2016, and 2021, when the incidence of enterovirus HFMD was very high. The impact of this type of enterovirus on various infectious diseases that were associated with EVA71. For patients diagnosed with meningitis or encephalitis and with cases of acute flaccid paralysis, the results were not as clear as for cases of enterovirus HFMD (Figure 25). Among the clinical forms of enterovirus infection associated with EVA71 in South Vietnam, the proportion of fatal cases was not the same, it was 21.9% among cases with a primary diagnosis of meningitis or encephalitis and 1.8% among cases of enterovirus HFMD (Table 21).

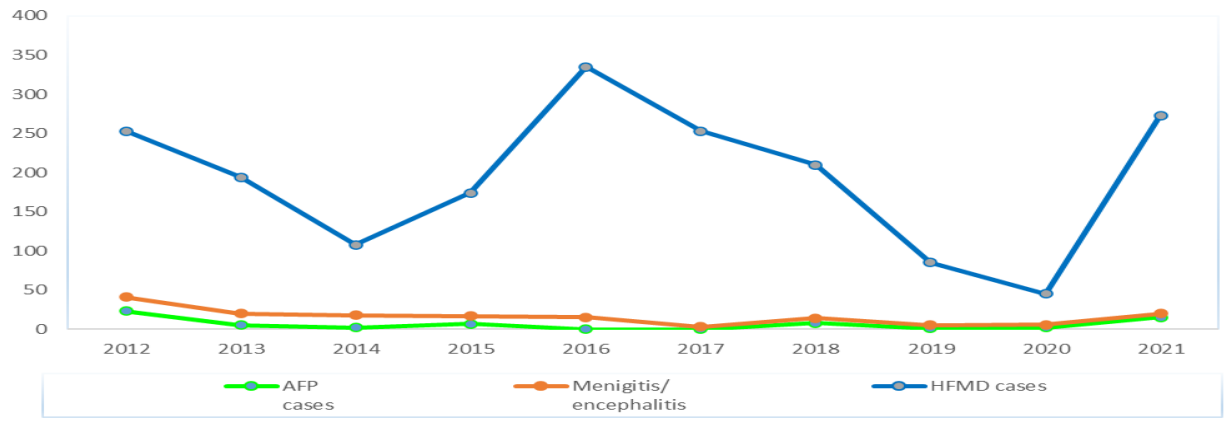

Figure 25. EVA71- associated cases of various clinical forms of enterovirus infection and acute flaccid paralysis in 2012-2021

Table 21

EVA71 strains isolated from patients with various infectious diseases in 2010-2021

Year	**AFP cases**	**Meningitis or encephalitis cases**			**HFMD cases**			
		Alive	Fatal	total	Mild	Complicated	Fatal	total
2010	1							
2021	3							
2012	23	11	7	18	10	183	19	212
2013	5	10	5	15	2	164	8	174
2014	2	13	3	16	14	76	0	90
2015	7	9	1	10	21	136	0	157
2016	0	15	0	15	0	320	0	320
2017	0	3	0	3	0	250	0	250
2018	8	2	4	6	11	183	2	196
2019	1	4	0	4	18	62	0	80
2020	2	3	1	4	0	39	0	39
2021	15	5	0	5	15	235	3	253
Total	**67**	**75**	**21**	**96**	**91**	**1648**	**32**	**1771**

Among 1,934 EVA71- associated cases with different clinical forms, the proportion of infants of less than six months was 2%, the share of children from 7 to 12 months was 18%, the children from 13 to 36 months represented 72% among the total number of patients and 10% of patients were from three to fourteen years of

age. The proportion of boys (62%) exceeded ($p < 0.05$) the same indicator for girls (38%), that can be seen from Table 22.

Table 22

Data of patients with various diseases from whom EVA71 strains were isolated in South Vietnam in 2010-2021

Diagnosis	Number of cases	Gender distribution		Age distribution			
		boys	girls	0-6 months	7-12 months	13-36 month	3-14 years
AFP	67	38	29	1	8	48	10
Meningitis	96	68	28	6	10	70	10
HFMD	1771	1093	678	66	254	1278	173
Total	1934	1199 (62%)	735 (38%)	73 (3%)	272 (15%)	1396 (72%)	193 (10%)

EVA71- associated infections were registered in all thee provinces throughout South Vietnam. Ho Chi Minh city showed the highest incidence of these infections, Dong Thap and Can Tho reported around 200-299 cases, An Giang and Tay Ninh – 100-199 cases, the remaining provinces had less than 100 cases (Figure 26).

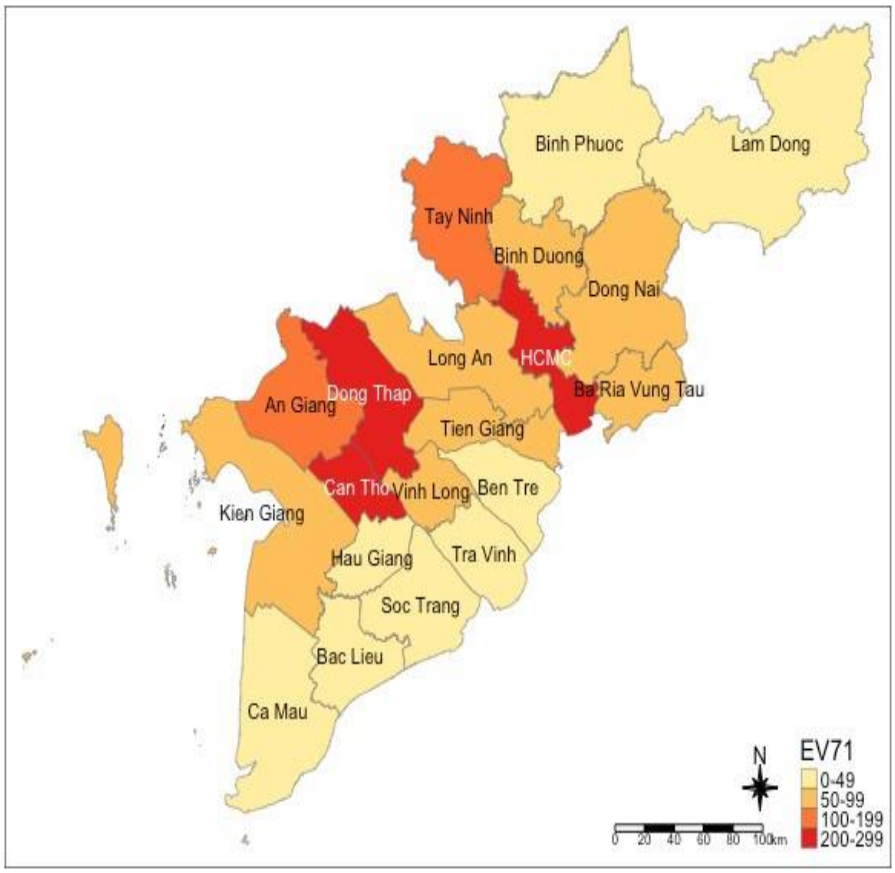

Figure 26. Geographic distribution of cases infected with EVA71 in South Vietnam 2010-2021.

The obtained results of investigations helped to determine the leading role of enterovirus 71 in circulation among the population of South Vietnam and also the leading role of this virus in the etiology of different clinical forms of nonpolio enterovirus infection. These results proved the necessity to force the effectiveness of control the circulation of enterovirus 71 in South Vietnam as well as to prognose the outbreaks of nonpolio enterovirus infection associated with enterovirus 71. The constant control of virus circulation and other prophylactic measures will be the new action for strengthening of national and regional programmes of surveillance and reinforcement of struggle with enterovirus infection.

3.4 MOLECULAR CHARACTERISTIC OF SOME ENTEROVIRUSES CIRCULATED IN SOUTH VIETNAM

3.4.1 Molecular characteristics of enterovirus 71 strains circulating in South Vietnam

Based on phylogenetic analysis of the VP1 region of the genome, Enterovirus 71 viruses are divided into six genotypes, A through F. Genotype A includes the prototype strain EVA71 (BrCr-CA-70), which was isolated in 1970 in the United States. Genotypes B and C can be further divided into the major genotypes B1-B5 and C1-C5, respectively. Recently, genotype D viruses have been identified in India, and genotype E and F strains have been identified in Africa.

Molecular analysis conducted in 2018-2019 showed that 88% of EVA71 strains isolated from patients with enterovirus HFMD and AFP in South Vietnam belonged to the C4 genotype, and 12% of EVA71 strains were represented by the B5 genotype. The complete nucleotide sequences of the VP1 genomic region for 67 EVA71 viruses were deposited in GenBank under accession numbers **MW139687-MW139744** and **OP947996-OP948003**. Phylogenetic analysis of the nucleotide sequences of the VP1 genomic region of the studied EVA71 strains of genotype C4 from Vietnam is show in Figure 27 and EVA71 strains of genotype B5, which formed one monophyletic group, are shown in Figures 28.

Nucleotide sequences of EVA71 strains detected in South Vietnam formed a monophyletic cluster with sequences of EVA71 strains circulating in Germany in 2018–2019 [131, 132] and caused a large outbreak of enterovirus exanthema in China in 2016-2018. Vietnamese EVA71 strains had 99.2-99.9% nucleotide homology with EVA71 strains detected in patients with enterovirus HFMD and AFP examined in 2017-2018 in the Chinese province of Yunnan.

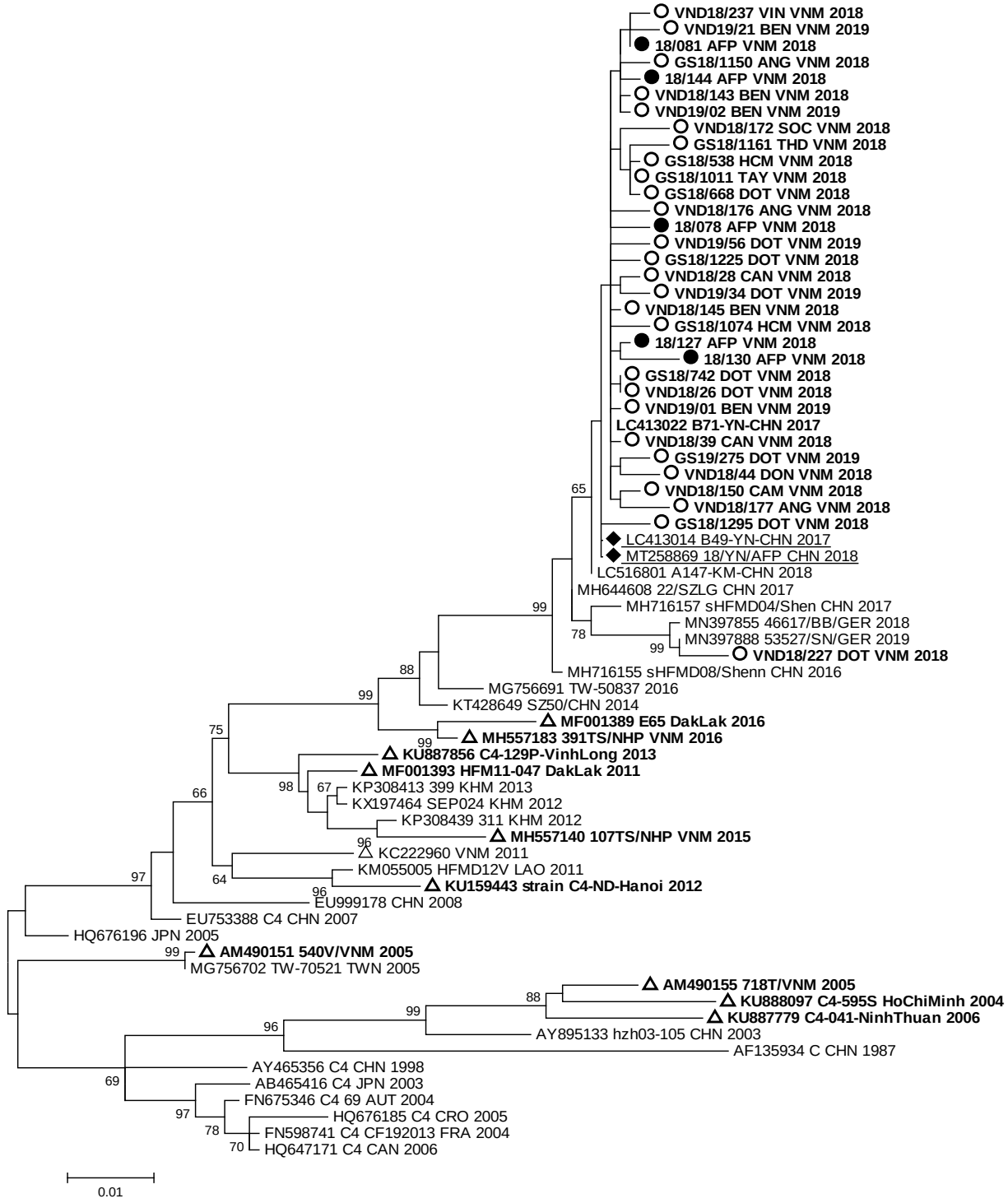

Figure 27. Phylogenetic relationships between EVA71 strains of genotype C4 identified in Vietnam: ○ - Vietnamese strains isolated from patients with HFMD; ● - Vietnamese strains isolated from patients with AFP; Δ - Vietnamese strains described in other studies; ♦ - strains identified in patients with AFP from the Chinese province of Yunnan.

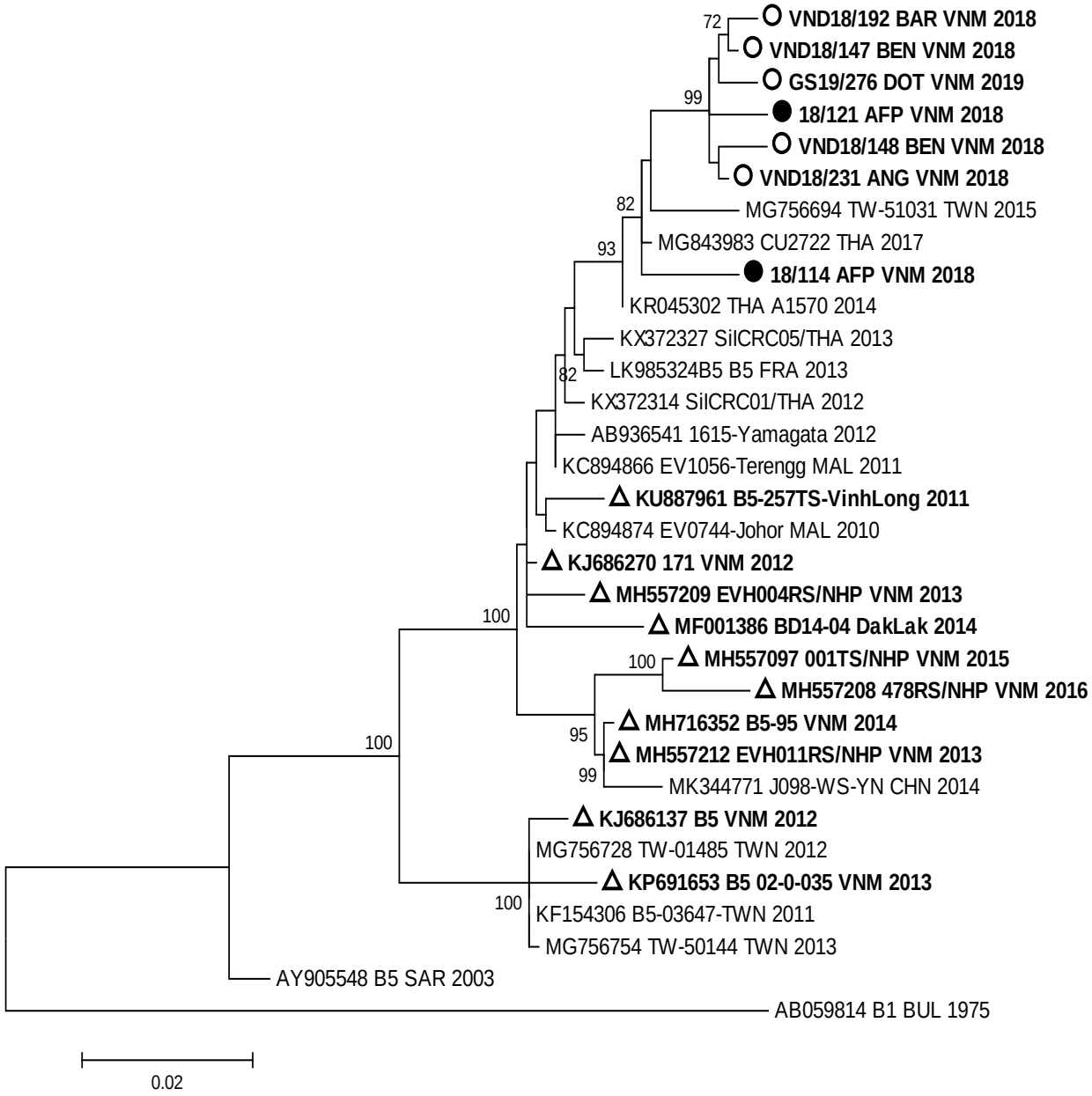

Figure 28. Phylogenetic relationships between EVA71 strains of genotype B5 identified in Vietnam: ○ - Vietnamese strains isolated from patients with HFMD; ● - Vietnamese strains isolated from patients with AFP; Δ - Vietnamese strains described in other studies.

The EVA71 genotype C4 strain VND18/227, identified in a patient with hand, foot, and mouth disease who had a complicated clinical course of the disease, was very similar to the EVA71 strains (99.2–99.4% homology) of genotype C4 identified in Germany. The remaining viruses represented a separate genetic group and were

highly related to the EVA71 strains detected in the Chinese province of Guangdong in 2017 [126]. The EVA71 genotype C4 viruses detected in our study in cases of hand-foot-and-mouth disease and AFP differed from those detected in 2003–2005 and 2011–2012 EVI outbreaks with clinical presentation of hand foot and mouth disease in southern and northern Vietnam [132, 136]. They also differed from the strains isolated in Dak Lak Province in 2016 [71, 135].

The sequences of the studied EVA71 genotype B5 strains, which were isolated and/or identified in patients with hand-foot-and-mouth disease and AFP, formed a monophyletic group (Figure 28).

All genotype B5 strains showed close relationship with Chinese genotype B5 viruses isolated in 2017 [105], but they were distinct from the EVA71 genotype B5 viruses circulating in Vietnam in 2011–2016 [71].

3.4.2 Molecular characterization of enterovirus CVA10 strains circulating in South Vietnam

Enteroviruses of the Coxsackievirus A10 type also circulated frequently in Vietnam. Coxsackie A10 strains were the second most frequently detected viruses isolated from patients with acute flaccid paralysis syndrome (24 strains), after enterovirus 71, which comprised 67 strains. Among patients diagnosed with hand, foot, and mouth disease, Coxsackie A10 viruses (359 strains) were the third most frequently detected, after enterovirus 71 (1,771 strains) and Coxsackievirus A6 (830 strains).

The study analyzed the molecular characteristics of Coxsackie A10 strains identified in South Vietnam. These strains were compared with Coxsackie A10 strains identified in other countries of Southeast Asia, as well as in Russia. Coxsackievirus A10 strains identified in Russia belonged to genotypes C and F3. Circulation of Coxsackievirus A10 strains of genotype F3 in the Russian Federation was first documented in 2013, after which these strains circulated annually from 2015 to 2018.

Most strains identified in South Vietnam in patients diagnosed with acute flaccid paralysis syndrome or hand-foot-and-mouth disease were found to belong to

genotype F3. Only one strain, isolated from a patient with severe neurological complications of hand-foot-and-mouth disease, was classified as genotype F1 (Figure 29).

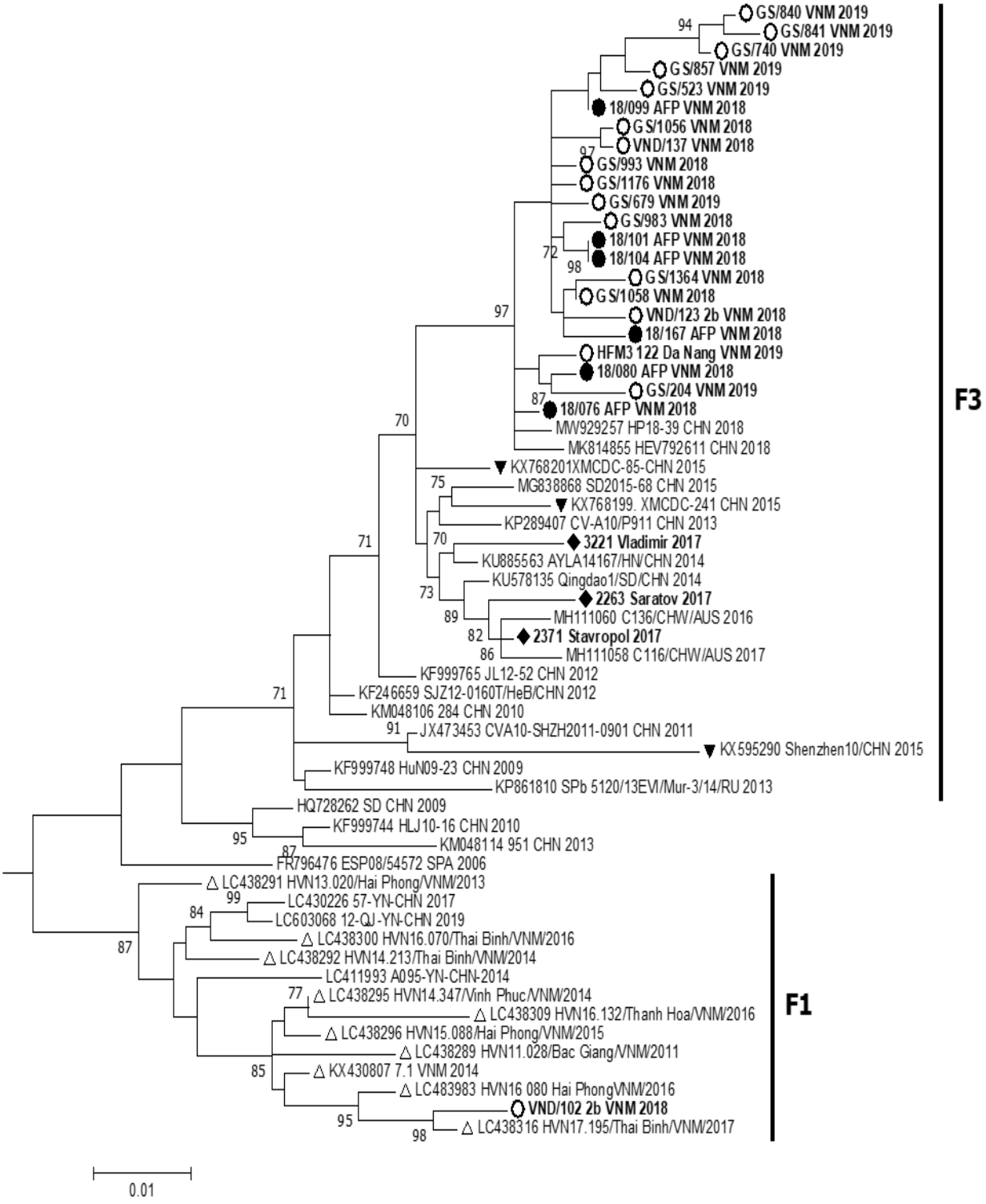

Figure 29. Phylogenetic relationships between Coxsackie A10 genotype F strains identified in Vietnam and other countries.

Within the F genotype, the studied strains were genetically heterogeneous. All Coxsackie A10 strains isolated in Russia and belonging to the F3 subgenotype of genotype F were represented by at least five genetic variants and showed a relationship with various strains circulating in Southeast Asia and the Pacific region, including viruses isolated from patients with severe forms and fatal cases of enterovirus infection [15].

Most of the strains from South Vietnam identified during joint Russian-Vietnamese studies formed a reliable phylogenetic group together with Chinese strains isolated in 2017-2018 in Yunnan Province. This group of strains included viruses isolated from patients with varying disease severity. There is no data on the circulation of CV-A10, belonging to this genetic variant, in Vietnam in previous years. The VND18/102 strain, isolated in 2018 from a single patient with enterovirus exanthema with severe neurological complications, showed a close genetic relationship with CV-A10 genotype F1 strains circulating in South and North Vietnam in 2014–2017 [42, 90].

DISCUSION

Analysis of the long-term dynamics of poliomyelitis incidence showed that periods of increased incidence alternated with periods of decreased incidence. In addition to epidemiological surveillance, the most important component of the implementation of the poliomyelitis eradication program is the virological study of biological samples from all the cases of AFP. This allows for the epidemiological and virological classification of each case of the disease [123].

In a virological study of biological material samples from 2,143 patients with a clinical diagnosis of acute flaccid paralysis, the percentage of poliovirus isolation was 1.0%. Wild polioviruses were not detected during the observation period from 2010 to 2021. Among the 22 polioviruses isolated from patients, 20 viruses were Sabin strains or vaccine viruses according to the results of intratypic differentiation and two strains were classified as vaccine-derived polioviruses of type 2 (VDPV2). From 20 vaccine polioviruses, 11 strains (55%) were classified as type 3, six strains belonged to type 1 (30%) and three strains (15%) were classified as type 2. A similar result was obtained in another country in Southeast Asia 43.8% > 34.3% > 21.9% [119].

During the period of study of poliovirus circulation in the country, no cases of vaccine-associated poliomyelitis (VAPP) were fixed. But in 2012, two cases of acute flaccid paralysis caused by vaccine-derived polioviruses VDPV2 were registered. Two localized cases associated with type 2 VDPV occurred due to violations in vaccination in two remote areas. One area was characterized by a humid tropical climate and poor living conditions, where the Khmer population, who speak other language, made up more than 80%. The second area was a hard-to-reach mountainous area of Dong Nai province, where people from 11 small ethnic groups live. All these factors, which impede the provision of high-quality vaccination services, and inadequate sanitation conditions created conditions for the spread of polioviruses excreted into the environment by recently vaccinated children. According to WHO, VDPVs have been detected in healthy people and in the environment in 13 other countries [11].

Widespread use of oral poliovirus vaccine has helped eradicate poliomyelitis in many countries around the world, reducing the spread of this disease. However, the use of OPV, which consists of live attenuated polioviruses, can lead to the formation of VDPVs. During circulation among the population, vaccine polioviruses can rise the neurovirulence and ability of transmission to other individuals of neurovirulent polioviruses. Modified viruses can cause paralytic diseases in unvaccinated children with compromise immunity [25, 146]. The rate of spread of VDPVs is similar to that of wild polioviruses [146]. Low levels of herd immunity against poliomyelitis, especially in rural, remote and isolated areas, was the main reason for formation of vaccine-derived polioviruses [28].

Immunization of children with poliovirus vaccines plays a key role in the implementation of the Polio Eradication Program. Polio vaccination is currently ongoing, as the infection has not been eradicated globally [108]. Until 2016, children in Vietnam were vaccinated with the trivalent poliovirus vaccine (bOPV) at 2, 3, and 4 months of age. Since June 2016, a bivalent poliovirus vaccine (bOPV), which includes poliovirus types 1 and 3, has been used. In September 2018, inactivated poliovirus vaccine (IPV) was introduced into the expanded immunization program in Vietnam for the fourth dose of vaccination at 5 months of age.

The acute flaccid paralysis syndrome surveillance system in South Vietnam strictly followed WHO recommendations and national guidelines, ensuring reliable AFP surveillance. At the same time, the standard WHO target for AFP case detection and the polio vaccination algorithm were strictly adhered to, vaccinating more than 90% of children under one year of age. These results demonstrate the effective maintenance and preservation of South Vietnam's polio-free status.

It is important to note that in patients with AFP syndrome (2,143 cases), the percentage of non-polio enteroviruses isolated (11.6%) was much higher than the percentage of polioviruses detected. This is due to the high level of circulation of non-polio enteroviruses in the south provinces of Vietnam and the virologic and molecular characteristics of some of these viruses. For example, in mild cases of HFMD caused by enterovirus 71, the disease can be complicated by clinical

manifestations of acute flaccid paralysis against the usual typical rash on the oral mucosa and skin of the hands and feet. When such children were admitted to hospitals, where they were registered as patients with a primary diagnosis of acute flaccid paralysis syndrome.

The identified NPEVs belonged to 34 types of enteroviruses. The largest proportion of viruses (52%) had enteroviruses of A species, while the proportion of enterovirus 71 was 28% of all the enteroviruses identified in patients with AFP. In other countries of the Asian region, enterovirus EVA71 was also the most frequently detected virus in the course of AFP surveillance.

According to WHO data, about 10% of viruses isolated during AFP surveillance are identified as NPEV. This index is used to assess the viability of the virus in stool samples during transport to the laboratory [96]. The percentage of NPEV detected in patients in this study was higher than the percentage detected in 2014 by Hong Kong National Certification Committee for Wild Poliovirus Eradication report [86].

Epidemiological and virological surveillance of poliomyelitis, as well as surveillance of enterovirus infection, is one of the most important tasks for Vietnam. The purpose of surveillance of EVI is to obtain information on the changes in the epidemic situation, clinical manifestations and the molecular evolution of etiologic agents, which can ultimately ensure control of EVI incidence.

The incidence of enterovirus infection was not seasonal, since the climate in the southern provinces of Vietnam is hot and humid throughout the year. At the same time, the incidence of EVI in different provinces fluctuated in different periods. Analysis of clinical picture of the disease indicates the leading role of such a clinical form as enterovirus exanthema of oral cavity and extremities – HFMD, which often occurred with neurological complications. The share of this clinical form in the structure of all forms of EVI was very high and fluctuated in different years from 58.3% to 98.9%.

A comprehensive approach to analysis of the problem of enterovirus infection in South Vietnam revealed a clear predominance of HFMD forms of infection and showed that the main etiologic agent of this disease was enterovirus 71. Strains of

this pathogen were identified in patients with various infections (acute flaccid paralysis syndrome, enterovirus meningitis, HFMD and other enterovirus diseases).

From 2012 to 2021, 632,651 cases of enterovirus HFMD, including 104 fatal cases, were recorded in 20 southern provinces of Vietnam. The highest number of cases of virus exanthema of the oral cavity and extremities and fatalities were recorded in 2012. From 2012 to 2016, only hospitalized patients were recorded, while from 2017 to 2021, both inpatients and outpatients were recorded. In 2018, a new peak in enterovirus HFMD and fatalities associated with EVA71 was recorded fatalities [24, 46].

The results of the conducted studies indicate the clear leadership of enterovirus 71 as the etiologic agent of enterovirus HFMD in South Vietnam. The prevalence of EVA71 viruses among the studied strains determined the severity of the disease in most of the examined patients and explained multiple complications of the infection. Most cases of enterovirus exanthema of the oral cavity and extremities (78%) were registered in three provinces located in the Mekong Delta, two of which formed a single epidemiological focus of enterovirus HFMD [41].

The Mekong River has a significant impact on the epidemiology of enterovirus infection in southern Vietnam, as more than 20% of the total population of Vietnam lives in the Mekong Delta [113]. The river carries water mixed with wastewater from five countries to the southern provinces of Vietnam. This complicated the epidemic process and contributed to the high incidence of enterovirus exanthema in southern Vietnam. The hot and humid climate, high population density with a large proportion of children, and poor sanitation also negatively affected the incidence of enterovirus infection [114]. The year-round widespread circulation of non-polio enteroviruses among the population of southern Vietnam contributes to the activation of evolution of local and imported strains of enteroviruses, which usually leads to the formation of new virus variants with altered properties and often increased virulence and transmissibility [24, 41, 131].

The proportion of children under three years of age is 90% of the total number of children with EVI, among them the proportion of children from 13 to 36 months

was 72%. It should be emphasized that a high proportion of enteroviruses 71 was detected in the analysis of all clinical forms of enterovirus infection in South Vietnam [114]. Strains of EVA71 were isolated from all categories of examined patients, not only from patients with the HFMD form of infection, but also in cases with enterovirus meningitis or meningoencephalitis. The presence of strains of EVA71 in the biological material of patients diagnosed with acute flaccid paralysis, including acute flaccid myelitis, was confirmed by virological and molecular methods. Among the identified viruses found in patients with enterovirus HFMD, in patients with enterovirus meningitis and in patients with AFP, the proportion of enterovirus 71 was 49%, 42% and 28%, respectively. In all variants of enterovirus infection and in AFP, the proportion of boys (62%) statistically exceeded ($p \leq 0.05$) the proportion of girls (38.7%).

Enterovirus EVA71 was clearly prevalent in different variants of infectious diseases, and it is undoubtedly the leading enterovirus in circulation among the population in all provinces of South Vietnam in all categories of diseases studied. This proves the need to strengthen measures to reduce such a wide circulation of enterovirus 71 using all possible preventive measures.

According to molecular studies, 88% of the identified EVA71 strains were classified as genotype C4, and 12% belonged to genotype B5. The results of phylogenetic analysis of enteroviruses indicate the influence of the largest Asian waterway, the Mekong River, this densely populated basin, which forms a unique epidemiological focus of enterovirus infection, including cases with clinical features of enterovirus HFMD. This study established a very close relationship between most of the studied Vietnamese EVA71 strains of genotype C4 and viruses of the same genotype that circulated in the Chinese province of Yunnan, located in the upper reaches of the Mekong. Strains of EVA71 genotype B5 were genetically close to strains from Thailand, through which the Mekong also flows. Phylogenetic relationships analysis of enterovirus 71 strains from Vietnam indicated multiple cases of importation of this virus from neighboring countries and its wide spread within Vietnam. This led to high and stable incidence of HFMD form of infection up to the

present time. It is important to note that among the enteroviruses isolated from patients with AFP, there were strains belonging to both EVA71 genotypes – C4 and B5. These strains were not genetically different from strains isolated from patients with enterovirus exanthema of the oral cavity and extremities [114].

Hand, foot, and mouth disease is the most common enterovirus infection in Southeast Asia [41, 105]. Over the past 25 years, numerous outbreaks of HFMD with severe clinical manifestations, associated with enterovirus EVA71, were reported in Asian region – in Taiwan, China, and Vietnam (mainly South Vietnam) [87, 126, 131, 132]. Almost all studies indicate that this infectious disease in children often occurs with various neurological complications. HFMD primarily associated with enterovirus 71 remains a pressing public health problem in Vietnam, especially in South Vietnam. EVA71-associated infection has led to serious complications such as enterovirus meningitis/meningoencephalitis, acute flaccid paralysis, and even fatal cardiopulmonary collapse. In Vietnam, large outbreaks of this infection were recorded in the 21st century (2011–2012 and 2020–2021), resulting in thousands of hospitalizations and deaths among children under five years of age [40, 88], with children under 12 months of age particularly affected.

Several inactivated vaccines against EVA71-associated infections have been developed and approved worldwide [19]. In China, three inactivated vaccines derived from EVA71 genotype C4 are used, which have demonstrated greater than 90% efficacy against enterovirus 71-associated hand, mouth, and foot ulcers in children aged six to 35 months [73, 79, 150, 151], although infants under six months, who represent the highest-risk group, were excluded from the study. The EVA71vac vaccine, developed in Taiwan, expands the age range of its use, covering children aged two to six months. The inactivated EVA71vac vaccine, based on enterovirus 71 genotype B4, was developed and produced in Taiwan [16]. The B4 genotype-based EVA71vac vaccine showed in vitro cross-reactivity against genotypes B5, C4, and C5 [89]. The EVA71vac vaccine demonstrated good protective efficacy in trials conducted in Taiwan and in targeted use in South Vietnam during an outbreak of hand-foot-and-mouth disease. Twenty-two laboratory-confirmed cases of hand-foot-

and-mouth disease associated with enterovirus 71 were identified. These infections occurred only in unvaccinated children. However, no children vaccinated with EVA71vac were affected. Molecular analysis revealed that 11 cases were caused by EVA71 genotype B5 and 10 cases were associated with EV71 genotype C4, both of which differ from EVA71 genotype B4. Children aged two to six months accounted for 23% of patients diagnosed with hand-foot-and-mouth disease associated with enterovirus 71. Previous studies in Taiwan have shown that EVA71vac induces neutralizing antibodies with cross-protection against EVA71 genotypes B4, B5, C4, and C5 [51].

The EVA71vac vaccine is the first vaccine studied in infants aged two to six months, which is particularly relevant for Vietnam, where infants under six months of age, lacking maternal antibodies [133], are at high risk of severe outcomes if infected [117]. Including EVA71vac in the National immunization schedule in Vietnam could yield significant public health benefits by reducing severe cases, hospitalizations, and deaths, especially during peak epidemic seasons. This will reduce the economic burden on families and the healthcare system. Studies have shown that a mild case of EVA71-associated hand, mouth, and mouth disease in Vietnam costs an average of US$400. The average cost of hospitalization for complications of EVA71-associated infection is significantly higher. For example, the cost of treating encephalitis in children is US$1,859 [52, 91]. A study by the Asia Pacific Pediatric Association noted that from 2000 to 2020, EVA71-associated hand, mouth, and mouth disease outbreaks in the region were not only frequent but also increasingly severe, with EV71 enteroviruses of the C4 and B5 genotypes most commonly associated [17, 106]. Given the high efficacy of EV71vac in preventing such cases, vaccination may prove not only necessary to save children's lives but also cost-effective. The role of enterovirus EVA71 as an etiologic agent of infectious diseases in South Vietnam is critically important, it is associated with various clinical forms of enteroviral infection, often severe. This virus is the primary etiologic agent of the most common infectious disease in Vietnam: HFMD. The leading role of enterovirus EVA71 in circulation among population underscores the need for enhanced surveillance and

monitoring of enterovirus EVA71 circulation to predict outbreaks of hand, foot, and mouth disease, as well as to improve the prevention of non-polio enterovirus infection through vaccination against diseases associated with EVA71.

The study showed that in South Vietnam, the main etiologic agents of enterovirus HFMD and other forms of enterovirus infection, including AFP syndrome, were enteroviruses EVA71 (mainly genotypes C4 and B5). Since the EVA71 strains that caused cases with acute flaccid paralysis syndrome were genetically indistinguishable from the strains isolated from patients with enterovirus exanthema, it can be assumed that vaccination with EV71vac will affect the incidence rates of different forms of enterovirus infection, including the incidence of AFP. The results of the study provided important information on the circulation of etiologic agents of such a widespread infection in the country. They helped to understand the patterns of development of the epidemic process in different forms of enterovirus infection.

The data obtained once again emphasize the crucial role of active epidemiological and virological surveillance in informing health authorities of the actual epidemiological situation and enabling appropriate action. The primary goal of comprehensive anti-epidemic measures is to comprehensively reduce the incidence of infectious diseases, as well as the economic burden of non-polio enterovirus infection for South Vietnam and the country as a whole. To improve epidemiological surveillance, it is necessary to analyze the long-term incidence of various clinical forms of enterovirus infection. This requires assessing the impact of risk factors and the underlying causes of infectious disease outbreaks. A key component in the fight against infectious diseases is high-quality laboratory diagnostics, particularly high-quality virological testing, for accurate diagnosis and selection of appropriate treatment. Equally important is virological monitoring of the circulation of non-polio enteroviruses, which are often the etiologic agents of infectious diseases, among the population.

CONCLUSION

Since the certification of polio eradication, Vietnam has maintained its polio-free status. The quality of epidemiological and virological surveillance for acute flaccid paralysis syndrome meets all requirements set by national and international polio surveillance systems. All cases of AFP in South Vietnam were identified, reported, promptly investigated, and laboratory tested. To prevent the risk of vaccine-associated paralytic poliomyelitis, it is essential to maintain polio vaccination coverage among children at a level of at least 95%, emphasize the benefits of polio vaccination to the public, and strictly adhere to sanitary legislation when vaccinating children. AFP surveillance is essential, as wild polioviruses can be imported and circulate in polio-free countries if vaccination coverage declines. The goal of surveillance is to detect and assess the circulation of imported wild polioviruses and vaccine-derived polioviruses with increased neurovirulence and transmissibility. Identification of such pathogenic viruses is based on the analysis of poliovirus strains isolated during AFP surveillance using virological and molecular methods. Large-scale and safe polio vaccination helps prevent the circulation and transmission of wild polioviruses and vaccine-derived polioviruses imported into polio-free countries to susceptible individuals. Effective vaccination, along with high-quality surveillance, has been and will remain the key to Vietnam's epidemiological well-being.

Hand, foot, and mouth disease is a critical public health issue in Vietnam. Since the early 21st century, outbreaks of this disease (mainly associated with enterovirus EVA71) have been cyclically reported in Vietnam. Systematic monitoring of the circulation of EVA71 and other non-polio enteroviruses in Vietnam is a means of tracking viral evolution, including the emergence and spread of new non-polio enterovirus variants, to predict changes in the epidemic situation and develop adequate preventive strategies to combat infections. The need to reduce the economic and social impact of non-polio enterovirus infection requires more effective prevention and improved vaccination policies. Protection against non-polio enterovirus infection in Vietnam through vaccination against infections associated with its main etiologic agent, EV71, is essential. Vaccines against EVA71, which

exist and are produced in Southeast Asia, can serve as a valuable tool for combating infections associated with enterovirus 71. The use of such vaccines could become a key element in the fight against infectious diseases, helping to address the critical issue of enterovirus infection control in Vietnam.

CONCLUSIONS

1. From 2143 patients with acute flaccid paralysis syndrome during 12 years 22 poliovirus strains (1%) were isolated. Only two of them were vaccine-derived polioviruses – VDPV2, that proved high quality surveillance and vaccination policy in South Vietnam. The isolation from this patients 11.6% of non-polio enteroviruses largely circulated in South Vietnam connected with severe neurologic complications of non-polio enterovirus infection.

2. The long-term analysis of non-polio enterovirus circulation revealed the domination of species A enteroviruses (alphacoxsackie), these 3798 strains constituted 93% of isolated enteroviruses. Etiological association of species A enteroviruses with HFMD caused the leading role of this clinical form of non-polio enterovirus infection in South Vietnam. Species B enteroviruses (betacoxsackie) were more often found in patients with enterovirus meningitis.

3. Enterovirus 71 was the main etiological agent of non-polio enterovirus infection in South Vietnam including its leading form HFMD. EVA71 strains were detected in patients with different clinical diagnosis: in 49% of HFMD patients, in 42% of enterovirus meningitis patients, and in 28% of acute flaccid paralysis patients. The changes in enteroviruses types and genotypes led to periodical peaks of enterovirus infection incidence in South Vietnam.

4. It was revealed that associated with enterovirus 71 HFMD cases in South Vietnam often complicated by severe neurological symptoms as meningitis and acute flaccid paralysis in early stages of infection were registered and hospitalised under the diagnosis enterovirus meningitis or acute flaccid paralysis.

5. Molecular analysis showed that 88% of studied enterovirus 71 strains belonged to the C4 genotype, and 12% of enterovirus 71 strains were representatives of the B5 genotype, enteroviruses of both genotypes largely circulated in South Vietnam. Most studied Coxsackievirus A10 strains belonged to the F3 genotype, and one Coxsackievirus A10 strain belonged to F1 genotype.

LIST OF ABREVIATIONS

VAPP- vaccine-associated paralytic poliomyelitis

WHO - World Health Organization

ITD – intratypic differentiation

WPV – wild poliovirus

IPV - inactivated poliovirus vaccine

NL - National Laboratory (polio)

nOPV2 - new oral poliovirus vaccine type 2

AFP - acute flaccid paralysis (syndrome)

OPV – oral poliovirus vaccine

PV - poliovirus

VDPV – vaccine-derived poliovirus

PCR - polymerase chain reaction

cVDPV2- Circulating vaccine- derived poliovirus type 2

EV - enterovirus

NPEV - non-polio enterovirus

EVI - enterovirus infection

EVM - enterovirus meningitis

HFMD - hand, foot and mouth disease

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