# Clinical manifestations, diagnosis, complication and management of enterovirus 71encephalitis.

*Thesis*

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# اعراض و طرق تشخيص وعلاج الإصابة بانتيروفيرس٧١المسبب لالتهاب الدماغ.

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# ABSTRACT

**Background**: Acute encephalitis is relatively uncommon but potentially detrimental CNS inflammation usually caused by infection. Enteroviruses have been reported in encephalitis cases. However, clinical and epidemiological characteristics of Enteroviruses in encephalitis are not fully established.
This study aimed at investigating the presence of Enterovirus in the CSF of encephalitic patients using PCR together with the clinical spectrum of the disease

**Methods:**
44 children with encephalitis were prospectively investigated over a period of 16 months. All patients were subjected to CSF examination using real-time PCR (RT-PCR). Demographic and clinical data were collected from the patients.

**Results:**
The age range of the patient was 6 months – 12 years (40.25 $\pm$ 38 months) mean $\pm$ sd. Most infections occurred during the warm months of the year.

Enterovirus was detected in 4 specimens (9.1%). (25.0%) of affected patients were preceded by gastroenteritis symptoms. The most frequent neurological manifestations were convulsion in (75.0%), altered mental state in (68.2%), brain stem affection in the form of apnea in (25.0%) and acute flaccid paralysis in (13.6%) of cases. 75% of the patients showed abnormal CT brain finding. 25% of cases received intravenous immunoglobulin's .all patients were managed in intensive cares and (25.5%) received mechanical ventilation. Short term sequalae occurred in the form of motor deficits and lower limb weakness in 25% of cases. And mortality rate occurred in 75% among EV affected patients.

**Conclusions:** RT- PCR is useful in detecting early EV encephalitis.
Enterovirus is a significant cause of encephalitis in younger age children with serious neurologic manifestations and high fatality rate.

**Keywords:** Enterovirus, encephalitis, pediatrics, RT-PCR

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| ANS | autonomic nervous system |
| ATP | Adenosine triphosphate |
| AFP | Acute flaccid paralysis |
| BBB - | blood-brain barrier |
| BE | brain stem encephalitis |
| Ca | calcium |
| CNS | Central Nervous system |
| CPF | cardiopulmonary failure |
| CR2 | Complement receptor 2 |
| CRRT | Continuous renal replacement therapy |
| CSF | cerebrospinal fluid |
| CT scan | Computerized tomography scan |
| CV | Coxsackieviruses |
| CVVH | Continuous veno-venous hemofiltration |
| CXC | Chemokine family |
| DNA | Deoxyribonucleic acid |
| ECLS | Extracorporeal Life Support |
| ECV | echovirus |
| EEG | Electro Encephalography |
| ELISA | Enzyme-linked immunosorbent assay |
| EV 71 | Enterovirus 71 |
| HEV | Human Enterovirus |
| HFMD | Hand Foot Mouth Disease |
| HIV | Human Imunodefienicy disease |
| HLA | Histocompatibility leukocyte antigen |
| HR | Heart Rate |
| IC | Internal control |
| IFN | interferon |
| IgG | immunoglobulin G |
| IgM | immunoglobulin M |
| IL | interleukin |
| IP-10 | Induced protein-10 |
| IVIg | Intra-venous immunoglobulin |
| LP | lumbar puncture |
| LV | left ventricular |
| MCP | monocyte chemoattractant protein |
| MHC | major histocompatibility complex |
| MIG | monokine induced by IFN-gamma |

# LIST OF ABBREVIATIONS (CONT.)

| | |
|---|---|
| MRI | magnetic resonance imaging |
| MV | Mechanical ventilation |
| Na | sodium |
| NK cells | natural killer cells |
| PCR | Polymerase chain reaction |
| PE | pulmonary edema |
| PICU | Pediatric intensive care unite |
| PSGL-1 | P-selectin glycoprotein ligand- 1 |
| PV | Polioviruse |
| Qalb | albumin concentration quotient |
| K | potassium |
| RNA | Ribonucleic acid |
| SBP | systemic blood pressure |
| SCARB2 | scavenger receptor B2 |
| SVR | systemic vascular resistance |
| TGF-β1 | Tumor growth factor |
| TLC | Total leukocyte count |
| TNF | tumor necrosis factor |
| USA | Unit ate State of America |
| UTR | Untranslated region |
| VC | Virus culture |
| VLP | Virus-like-particle |
| VP | viral protein |
| VPg | virus encoded protein |
| WBC | White blood cell |
| WHO | World Health Organization Regional Office |

# INTRODUCTION AND AIM OF THE WORK

A global campaign has all but eradicated poliomyelitis from Europe, the Americas, and much of Africa and Asia Over the past 10 years, however, the related enterovirus 71(EV71) has emerged across Asia, where it threatens to become what has been coined the new polio The virus is a member of the enterovirus genus, which includes coxsackieviruses and echoviruses (**Ooi et al, 2010)**.

Enteroviruses are small, single-stranded, positive-sense RNA viruses from the enterovirus genus in the family Picornaviridae They cause disorders with a wide range of clinical manifestations, including cutaneous, visceral and neurological diseases. For many years polioviruses were the most important enteroviruses, since they led to large outbreaks of paralytic disease (**Solomon et al, 2010)**.

Hand, Food and mouth disease (HFMD) is a communicable disease affects children. It is frequently associated with enterovirus EV71 and its main clinical features include rashes in hand, food, or mouth, aseptic meningitis, poliomyelitis-like, acute flaccid paralysis, brainstem encephalitis, and other severe systemic disorders, such as pulmonary edema and cardiorespiratory collapse **(Tian et al, 2012).**

The HFMD is considered a self-limiting illness and most children who present with aseptic meningitis generally have good outcomes. However, a small proportion can rapidly develop neurological and systemic complications that can be fatal **(Tian et al, 2012).**

The clinical course of these infections has been staged according to the clinical course and the severity from stage I through III **(Lin et al, 2002)**.

However, disease progression from the onset of CNS involvement to acute cardiopulmonary failure may be extremely rapid. Admission to a hospital for close monitoring and prompt intensive care is usually mandatory in patients with CNS involvement. Nonetheless the clinical manifestation of CNS involvement may be subtle, especially in young children with early-stage disease (**Lin et al,2006**).

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# AIM OF THE WORK

The aim of this study is:

1. In this Review we discuss the clinical and molecular epidemiology, pathogenesis
2. Clinical features, diagnosis, and treatment of EV71 disease.

# ENTEROVIRUS 71

## Virology

## Classification

As well as the enterovirus genus, the large Picornaviridae family include Rhinovirus spp (eg, the common cold), Hepatovirus spp (eg, human hepatitis A virus), Parechovirus spp (eg, human parechovirus 1 and 2), and two important animal virus genera, Cardiovirus spp (eg encephalomyocarditis virus) and Aphthovirus spp (foot and mouth disease virus) **(Pallansch et al,2001**).

Human enteroviruses were traditionally separated into four classifications, according to their pathogenicity in human beings and experimental animals and their cytopathic effects in tissue culture these subgroups were polioviruses (three serotypes), coxsackievirus A (23 serotypes), coxsackievirus B (six serotypes), and echoviruses (28 serotypes) **( Pallansch et al,2001**).

However because of the limitations of this system, serologically distinct human enteroviruses isolated since 1970 have been designated by serotype numbers, beginning with HEV68. The original classification of human enteroviruses has been substituted by a taxonomic scheme based on molecular and biological properties of the viruses **(Nasri et al,2007)**.

This revised classification recognizes at least 90 subtypes and separates them into four species. Polioviruses have been designated as members of the human enterovirus C species because they are genetically closely related **(Brown et al, 2003).**

**Physicochemical properties**

The virus capsid comprises 60 identical subunits (protomers), each of which contains a copy of the four structural viral proteins (figure1) **(Brown et al, 1995)**.

The lack of a lipid envelope confers human enteroviruses stability in the host environment, including on exposure to human gastric acid, and they can survive at room temperature for several days. EV71 and other enteroviruses have also been detected in surface and ground water and in hot spas **(Chen et al, 2008).**

Enteroviruses are resistant to organic solvents(eg.ether and chloroform), alcohol, and freezing, but can be inactivated by temperatures

higher than 56°C chlorination, formaldehyde, and ultraviolet irradiation In one study EV71 was destroyed by virucidal disinfectants**(Chan et al, 2005)**.

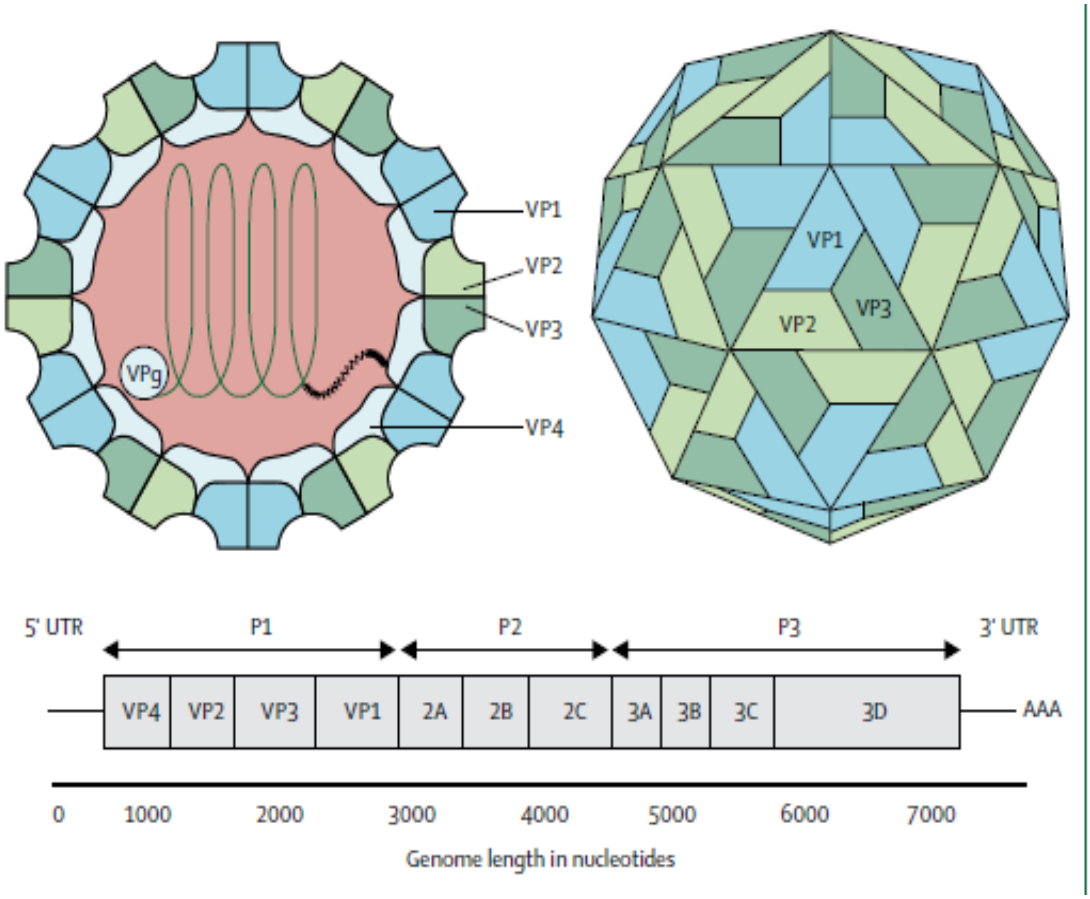


***Figure 1:***

**Enterovirus 71 structure and genome structure of the virion(Solomon et al,2010) UTR=untranslated region. VPg=virus encoded protein**

Human enteroviruses are small (circumference around 30 nm), non-enveloped, icosahedral particles that contain a single-stranded, positive-sense, polyadenylated virus RNA of approximately 7·4 kb. Each protomer in the virus capsid contains a copy of the four structural viral proteins (VP1–VP4), of which VP1, VP2, and VP3 are external whereas VP4 is completely internalized and is not, therefore, exposed to the host antibody response**(*Solomon* et al, 2010)**.

All the structural proteins are encoded by the P1 region of the genome The P2 and P3 regions encode seven non-structural proteins-2A-2C and 3A-3D. Reproduced from Viral Zone (**McMinn, 2002)** .

# Human EV71 Molecular Biology

At a molecular level, picornavirus infection is comprised of four processes: virus entry into cells, viral protein synthesis, viral RNA replication and virion as sembly and release. The virus must also escape host immune responses **(Bek and McMinn, 2012).**

## Transmission of EV71 and Entry into Cells

Humans are the only known reservoir for EV71; the faecal-oral route is the commonest mode of transmission, although respiratory droplet spread may also play a role **(Melnick, 1996)**. EV71 shedding in the stool was longer than in the throat **(Chung et al, 2001),** which may be for up to 42 days post-infection **(Han et al, 2010).**

During the EV71 outbreak in Taiwan in 2001-2002, the principal transmission was between children in childcare facilities. However, the transmission rate to household contacts was as high as 52% especially to siblings and cousins **(Chang et al, 2004).** Adults are rarely infected, as most adults already have previous immunity. However, infected adults may excrete the virus without signs and symptoms, and transmit virus to susceptible children **(Chan et al, 2011).**

EV71 has been isolated from worldwide HFMD outbreaks every year, suggesting the continuous circulation of the virus in the population. The persistence of enteroviruses has been well documented in the environment, such as in sewage and water systems **(Melnick, 1996),** and in spring water and environmental water in Taiwan **(Hsu et al, 2008).**

EV71 may also survive in the environment for at least three days at tropical room temperature **(Chan et al, 2005);** the persistence of EV71 in the environment may provide a continuing source of potential exposure for susceptible populations **(Chan et al, 2011)**.

A very low rate of enterovirus infections amongst blood donors has been documented, suggesting that blood components are unlikely to be an important route of transmission **(Welch et al, 2003).** Prenatal transmission of enterovirus infections are common, however in EV71 only one case has been documented **(Melnick, 1996**).

The first stage of virus entry is adsorption to the cell surface via an interaction with a specific receptor, followed by internalization and uncoating. HEV71 uses several different receptors to attach to cells (**Bek ,McMinn et al, 2010**), the most important being scavenger receptor B2 (SCARB2) **(Yamayoshi et al,2009**).

# Epidemiology

EV71 infection has the seasonal distribution of a peak in the spring and summer months. The transmission of enterovirus occurs within families, daycare centers, playgrounds and hospital nurseries. More children would be congregating in a limited space, which provides a readily available reservoir for the rapid circulation of the virus (**Ang et al, 2009**).

The fecal-to-oral route is considered a major transmission route. Long periods of viral shedding may account for the widespread transmission of EV71 **(Chang et al, 2004)**. **Chung et al (2001)** demonstrated that EV71 is excreted through the stool of infected patients for up to 6 weeks. The culture-positive rate of throat swabs was higher than that of rectal swabs in the patients **(Chang et al, 1999)**.

Viruses in throat through the saliva or respiratory droplets of patients may be transmitted during the acute stage of the disease **(Ho, 2000)** Although hand-washing precaution is important for the period of virus excretion through feces, it is not sufficient to limit the spread and transmission of the virus and to prevent further epidemics. Therefore, isolation of infected patients within single rooms with masks should be considered during hospitalization of patients (**Chang et al, 2004**).

A change in clinical presentation could coincide with a change in the viral transmission pathway an epidemiological parameters like crowding, lack of sanitation and climate, could enhance virus transmission. An increased susceptibility of the population to EV71 infection could be another explanation (**Cho, 2010).**

## Clinical epidemiology

### Initial identification

EV71 was isolated from the stool of a child aged 9 months with encephalitis, in California, USA, in 1969, although an earlier isolate has since been identified (**Van der Sanden et al, 2009**) Within 5 years small outbreaks of neurological infections, including encephalitis and aseptic