Enteroviruses

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Abstract

The enteroviruses comprise a large group of small RNA viruses that cause a broad spectrum of disease. Of the more than 100 types known to infect humans, only the three polioviruses have been successfully controlled by vaccination. Although vaccines are now also in development for enterovirus type 71, the large number of strains precludes this approach for most enterovirus types. Similarly, effective antivirals remain elusive to date. This review updates the current classification of enteroviruses, laboratory aspects of diagnosis and major clinical syndromes.

Keywords Aseptic meningitis; diagnosis; enteroviruses; myocarditis; poliomyelitis; respiratory infection

Key points

- Enteroviruses are a common cause of disease, and infection is frequently diagnosed in patients admitted to hospital, particularly young infants
- Laboratory diagnosis of enterovirus infection is achieved by detecting viral RNA in clinical specimens by polymerase chain reaction, and is helpful in excluding other treatable causes
- Enteroviruses are an important cause of meningitis, myocarditis, rash and respiratory illness

Introduction

The enteroviruses are a large group of non-enveloped, single-stranded RNA viruses within the picornavirus family (*pico* RNA virus = small RNA virus). They are spread predominantly via the faecal-oral route and initiate infection in the gastrointestinal tract. Despite this, they are not a significant cause of gastroenteritis, but cause a wide spectrum of disease presentation and severity including meningitis, respiratory illness, myocarditis, rash and neurological illness. Enterovirus types known to infect humans include the polioviruses, group A and B coxsackieviruses (CVA, CVB) and echoviruses. Since 1970, newly described enteroviruses have been sequentially numbered.

Classification

In the modern era, the classification of enteroviruses is based on similarity of genome organization and phylogeny (see www.picornaviridae.com). The enterovirus genus of the *Picornaviridae* now includes 13 enterovirus species (enterovirus A–J, rhinovirus A–C), each species including phylogenetically related enterovirus serotypes and genotypes. The rhinoviruses, which previously formed a separate genus within the *Picornaviridae*, are genetically similar to enteroviruses and have therefore been reclassified as three rhinovirus species within the enterovirus genus.

The genus includes bovine, porcine and simian enteroviruses (enterovirus E–J species), as well as human enteroviruses, which make up most of enterovirus A–D species. A number of viruses previously regarded as human enteroviruses are now classified as distinct genera within the *Picornaviridae*, again on the basis of genetic organization; the parechovirus genus includes human parechovirus types 1 and 2 (formerly enterovirus 22 and 23) as well as several more recently described parechoviruses. Meanwhile, the hepatovirus genus includes hepatitis A virus (formerly enterovirus 72) as its sole member. This review focuses on viruses classified within the enterovirus A–D species, traditionally regarded as human enteroviruses.

The new molecular taxonomy informs our understanding of enterovirus biology and evolution, while molecular epidemiology continues to provide invaluable information on progress of the World Health Organization (WHO) polio eradication initiative; it identifies reservoirs sustaining transmission of poliovirus, the source of viruses imported into areas where eradication has been achieved, and circulation of vaccine-derived polioviruses. Molecular epidemiology of enteroviruses has also recently been employed to understand the role of genetic recombination in generation of novel enterovirus strains, and its importance in understanding the emergence and spread of successive enterovirus outbreaks.[1]

For the purposes of laboratory diagnosis and clinical management, it is usually unnecessary to differentiate between different enterovirus types in the acute setting, as type identification does not usually influence management. However, genotypic characterization of enteroviruses found in clinical samples enables those countries that have achieved polio eradication to demonstrate their continuing capability to detect polioviruses (including vaccine-derived strains), which is a requirement for maintaining polio eradication status. Molecular typing also allows identification of novel or re-emerging enteroviruses.[2]

Diagnosis of enterovirus infections

Although no specific antiviral therapy is currently licensed for enterovirus infections, an aetiological diagnosis can nevertheless be valuable in differentiating enterovirus infection from other treatable conditions, and in recognizing outbreaks or potential for spread in the hospital environment. Traditional diagnostic methods based on isolating virus from clinical specimens in cell culture or serology have largely been replaced by nucleic acid-based detection based on polymerase chain reaction (PCR) analysis despite a lack of standardization of test methodology or testing algorithm. Compared with virus isolation, molecular diagnosis has a number of advantages:

- **Speed** – many modern laboratories can achieve same-day testing of clinical samples. Point-of-care testing solutions

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such as GeneXpert® (Cepheid) allow testing to be performed in clinical areas or specimen reception sites. Without delays arising from sample transfer to the laboratory, or the constraints of batch testing, results can be generated on demand in around 2.5 hours.[3]

- **Sensitivity** – enterovirus PCR is generally 10–1000-fold more sensitive than culture, and can detect viral RNA in samples that cannot yield infectious virus, including histologically preserved tissues.
- **Specificity** – most methods are designed to amplify regions of the viral genome that are highly conserved among different enterovirus types. Thus, a broad range of enteroviruses can be detected, while other viruses, including parechoviruses, are not. Rhinoviruses, however, may be detectable in generic enterovirus PCR assays. The ability to sequence PCR-amplified nucleic acid provides a further check on specificity, if required.
- **Quality** – the amenability of molecular testing to automation and standardization, together with the availability of external quality assurance test panels, provides the clinical customer with a high degree of confidence where these measures have been employed.
- **Flexibility** – PCR can detect virus in a wide range of clinical specimens, including cerebrospinal fluid (CSF), blood, urine, lower respiratory secretions, tissue samples, throat swabs and stool. Detection of virus in either of the latter two can reflect superficial, asymptomatic infection of the gastrointestinal tract and therefore provides only circumstantial evidence of aetiology.
- **Laboratory workflow** – many laboratories now employ moderate-throughput systems for automated nucleic acid extraction, robotic pipetting and closed-system real-time PCR testing, although fully automated, random access molecular testing is not yet available. Enterovirus PCR testing thus sits well within the wider context of diagnostic microbiology. In addition, the common methodology of molecular diagnosis facilitates a syndromic approach to diagnosis, in which enterovirus testing can be integrated into PCR test panels, multiplex PCR tests or microarrays; this allows testing for a range of microbes of relevance in a given clinical scenario.

Clinical spectrum of enterovirus infections

Enterovirus infection begins in mucosal tissue of the gastrointestinal tract, and in many cases infection remains localized to this site. However, viral replication in the gastrointestinal tract can lead to viraemia, resulting in a second round of replication in tissues of the reticuloendothelial system. This can lead to greatly amplified secondary viraemia, which disseminates infection to the spleen and lymph nodes, and subsequently to organs such as the heart, central nervous system (CNS) and skin.

Despite reaching these target organs, not all enterovirus infections are associated with symptoms. The host's genetic constitution and physiological features, such as age, sex and immune and nutritional status, influence the outcome of infection, and viral factors are also important. The fact that different enteroviruses recognize different cell surface receptors partly determines the types of cell or tissue that can be infected, and enterovirus types vary markedly in replication efficiency in different cell types. All these factors help to explain the remarkable diversity of enterovirus-related syndromes (Table 1). The most important are discussed below.

Aseptic meningitis

Enteroviruses are a common cause of aseptic meningitis, particularly in countries where mumps has been successfully controlled by vaccination. In temperate climates, the incidence is usually highest during the late summer and autumn, corresponding with the peak of enterovirus circulation (Figure 1). Enteroviral meningitis is usually benign; more severe encephalitis, meningoencephalitis or acute flaccid paralysis (AFP) occurs in a few patients. Enterovirus meningitis is common in infants <3 months of age; they typically present with fever and irritability. Up to 77% of enterovirus-positive CSF samples from neonates do not show pleocytosis.

Rapid diagnosis of enteroviral meningitis is achieved by isolating virus or detecting viral RNA in CSF. However, CSF PCR can be negative in a proportion of patients with evidence of enterovirus infection at peripheral sites or with positive blood PCR.[4] Enterovirus detection in these samples can thus be useful for virus surveillance and infection control. Rapid enterovirus diagnosis is valuable in differentiating viral meningitis from bacterial meningitis and herpes simplex encephalitis. Diagnosis of enteroviral meningitis may allow termination of unnecessary antimicrobial therapy, curtailment of additional investigations such as computed tomography, and quicker discharge from hospital or intensive care unit, with consequent savings in healthcare costs.[5]

Clinical entities associated with enterovirus infection

Organ system	Clinical entity	Main serotypes involved
CNS	Paralytic poliomyelitis	PV 1–3
	Aseptic meningitis	Numerous
	Encephalitis	Numerous
Cardiac and skeletal muscle	Myocarditis	CVB
	Dilated cardiomyopathy	CVB
	Pericarditis	CVB
	Bornholm disease	CVB
Skin	Hand, foot and mouth disease	CVA16, enterovirus 71
	Rash	Numerous
Mucous membranes	Herpangina	CVA
	Viral conjunctivitis	Numerous
	Acute haemorrhagic conjunctivitis	Enterovirus 70, CVA24
Respiratory tract	Summer cold	Numerous
	Non-specific febrile illness	Numerous
	Lower respiratory tract infection	Enterovirus D68

PV, poliovirus.

Table 1

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