

ENTEROVIRUS D68: EPIDEMIOLOGY OF A VIRUS THAT CAUSES PEDIATRIC INFECTIOUS DISEASE

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ABSTRACT

Introduction: Enterovirus D68 (EV-D68) is a respiratory virus that primarily affects children and has been associated with sporadic outbreaks of respiratory tract disease worldwide. This study aimed to carry out a literature review considering several aspects involving EV-D68 infection: epidemiology, molecular evolution, prevention, diagnosis, and treatment of this infection.

Methods: Data from 976 whole genome sequences (WGS) of EV-D68 collected between September 1977 to September 2022 showed substitution rates of 4.06×10^{-3} nucleotides per site per year (s/s/y). Phylogenetic tree of EV-D68 by clades (A1, A2, B, B1, B2, B3, and C) was performed.

Results: Literature reports that EV-D68 was discovered in 1962 after being isolated from respiratory specimens of children with pneumonia in the USA. However, in the recent molecular analysis from Nextstrain data, we observed that the time to the most recent common ancestor (tMRCA) of A1 was 2005-04-17 in the USA, A2 was 2003-12-23 in China, B was 2003-07-06 in China, B1 was 2010-03-21 in Vietnam, B2 was 2006-11-25 in Vietnam, B3 was 2011-01-15 in China, and C was 2000-06-27 in the USA. The immune response to EV-D68 involves both innate and adaptive immunity, with the production of neutralizing antibodies and activation of T cells playing crucial roles. Prevention strategies for EV-D68 include practicing good hand hygiene, respiratory etiquette, and avoiding close contact with infected individuals.

Conclusion: EV-D68 was originated in Canada in 1995 and spread to Europe, Asia, Oceania, Latin America, and Africa.

Keywords: *Enterovirus D68; Respiratory Tract Diseases; Molecular Evolution; Epidemiology.*

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INTRODUCTION

Enteroviruses are a large group of viruses that belong to the Picornaviridae family. They are single-stranded RNA viruses and are known to cause a wide range of human illnesses. Enteroviruses are classified into several species, including Enterovirus A, Enterovirus B, Enterovirus C, and Enterovirus D¹⁻⁵.

Enteroviruses are transmitted primarily through the fecal-oral route, although they can also be spread through respiratory droplets and direct contact with contaminated surfaces. They can cause a variety of illnesses, ranging from mild respiratory infections, gastrointestinal disorders, and hand, foot, and mouth disease to more severe conditions such as meningitis, encephalitis, myocarditis, and acute flaccid myelitis^{1,3-5}.

Among the different species of enteroviruses, Enterovirus D68 (EV-D68) has gained attention in recent years due to its association with respiratory illness outbreaks, particularly in children. EV-D68 typically causes symptoms similar to the common cold, including coughing, sneezing, runny nose, and fever. However, in some cases, particularly in individuals with pre-existing respiratory conditions, EV-D68 can lead to severe respiratory illness, requiring hospitalization⁵⁻⁹.

EV-D68 is a type of virus that belongs to the family Picornaviridae, specifically the Enterovirus genus. It was first identified in California, United

States, in 1962. EV-D68 primarily affects children and causes respiratory illness, ranging from mild cold-like symptoms to more severe respiratory complications.^{6,10} The virus is spread through respiratory secretions, such as saliva, nasal mucus, or sputum, when an infected person coughs, sneezes or touches contaminated surfaces. It can also spread through close contact with an infected individual. EV-D68 tends to be more common during the late summer and fall seasons^{6,10,11}.

For several decades following its initial discovery, EV-D68 remained relatively uncommon and caused only sporadic outbreaks. However, in recent years, there have been notable increases in the number of EV-D68 cases reported worldwide. The largest outbreak to date occurred in 2014 in the United States, where multiple states reported a significant number of cases, particularly among children with asthma or a history of adventitious sounds^{5,6,10,11}.

During the 2014 outbreak, EV-D68 gained widespread attention due to the severity of respiratory illness it caused in some individuals. Many children with EV-D68 infection experienced difficulty breathing, wheezing, and respiratory distress, leading to hospitalization in some cases. This raised concerns about the virus's potential to cause severe respiratory complications, especially in individuals with pre-existing respiratory conditions^{5-7,10}.

Since 2014, EV-D68 continues to circulate, and sporadic outbreaks have been reported in various countries. However, the frequency and intensity of outbreaks can vary from year to year. It is important to note that while EV-D68 can cause severe respiratory illness, most individuals who contract the virus experience mild symptoms and recover without complications^{5,7,10}.

Prevention and control measures for EV-D68 are similar to those recommended for other respiratory viruses. These include regular handwashing with soap and water, covering the mouth and nose when coughing or sneezing, avoiding close contact with sick individuals, and disinfecting frequently-touched surfaces. Individuals with asthma or other respiratory conditions should ensure their condition is well-managed and follow their healthcare provider's guidance^{5,6,10,11}.

The exact mortality rate associated with EV-D68 is challenging to determine as it varies depending on factors such as population demographics, healthcare access, and the availability of supportive care. Additionally, not all cases of EV-D68 infection are reported, which can affect the accuracy of mortality data. In a meta-analysis, was identified the highest EV-D68 prevalences were in hospital outbreaks, in developed countries, in children under 5, and in patients with acute flaccid myelitis and asthma-related diseases. Sporadic deaths linked can occur associated with severe respiratory EV-D68 infections. EV-D68 shows a low prevalence of current as opposed to

the existence of EV-D68 antibodies in almost all children. Therefore, highlight the need to implement and/or strengthen continuous surveillance of EV-D68 infections in hospitals and in the community for the anticipation of the response to future epidemics^{5,12}.

This study aimed to carry out a literature review considering several aspects involving EV-D68 infection: epidemiology, molecular evolution, prevention, diagnosis, and treatment of this infection.

METHODS

The keywords "Enterovirus D68 global distribution", "Enterovirus D68 prevalence", "Enterovirus D68 biology", "Enterovirus D68 molecular evolution", "Enterovirus D68 phylodynamics", "Enterovirus D68 phylogeography", "Enterovirus D68 immunology", "Enterovirus D68 diagnosis", "Enterovirus D68 prevention", "Enterovirus D68 therapeutics", were used with Boolean combinations. The literature search and relevance evaluation were conducted with the databases PubMed, Web of Science, and Google Scholar. Articles found were considered potential reference sources. Searches were performed up to early June 2023.

RESULTS AND DISCUSSION

Enterovirus D68 epidemiology

EV-D68 has been reported in many countries, including the United States, Canada, European countries, and parts of Asia. The frequency and intensity of outbreaks may vary from year to year and between regions¹²⁻²⁵. EV-D68 infections primarily occur during late summer and fall, typically peaking between August and October in the Northern Hemisphere. However, sporadic cases can be reported throughout the year.^{19,26} EV-D68 infections predominantly affect children, particularly those between the ages of 4 and 12 years. This age group is more susceptible to the virus, possibly due to lower pre-existing immunity^{19,26,27}.

EV-D68 is known for causing respiratory illness, ranging from mild respiratory symptoms resembling the common cold to severe respiratory complications. It can lead to wheezing, difficulty breathing, and in some cases, hospitalization. Children with asthma or a history of wheezing are at higher risk of developing severe symptoms^{19,26}.

EV-D68 outbreaks have been associated with clusters of cases in various settings, including schools, daycare centers, and communities. These outbreaks often draw attention due to an increased number of severe respiratory illnesses reported within a specific time frame.^{19,28} Enhanced surveillance systems are in place in many countries to monitor EV-D68 activity. Public health agencies and laboratories actively monitor

respiratory illness patterns to detect and investigate outbreaks, identify circulating strains, and track changes in the virus over time¹⁹. EV-D68 is part of a larger group of enteroviruses that show seasonal patterns. Other enteroviruses, such as enterovirus A71, coxsackievirus, and echovirus, may circulate during the same season and cause similar respiratory symptoms^{19,28}. This virus exhibits genetic diversity, with multiple distinct clades identified worldwide. Molecular characterization helps in understanding the virus's evolution, tracking its spread, and identifying potential changes in virulence or transmissibility^{19,27,29}.

Molecular evolution of Enterovirus D68

EV-D68 is a positive-sense, single-stranded RNA virus belonging to the Enterovirus genus within the Picornaviridae family. Its genome is approximately 7.4 kilobases in length and contains a single open reading frame (ORF) flanked by untranslated regions (UTRs) at the 5' and 3' ends^{6,7,19,27,30}.

EV-D68 exhibits genetic diversity, and different clades of the virus have been identified based on genetic variations in the VP1 gene, which encodes the major capsid protein. Multiple genotypes, such as clade A, B1, B2, and C, have been described, with varying distribution and prevalence in different regions and over time^{27,29,30}.

Phylogenetic analyses have revealed that EV-D68 is closely related to other enteroviruses, including human rhinoviruses (HRVs) and other enterovirus species, such as enterovirus A and enterovirus C. This suggests common ancestry and evolutionary relationships with these viruses^{6,7,29}. Recombination, a process where genetic material is exchanged between different viral strains, has been reported in EV-D68. Recombination events can lead to the emergence

of new clades and contribute to the genetic diversity of the virus. The precise impact of recombination on EV-D68 evolution and its implications for virulence or transmissibility are still being studied^{7,27,30,31}.

The evolutionary dynamics of EV-D68 are complex and influenced by various factors, including immune pressure, viral fitness, host range, and population immunity. Evolutionary changes can occur through genetic mutations, recombination events, and selection pressures, leading to the emergence of new strains or variants over time^{6,9,29-32}. Molecular analysis has shown that different EV-D68 clades have circulated globally, with some clades being more prevalent in certain regions or during specific outbreak periods. Genomic surveillance helps track the spread of different clades and understand their geographic distribution^{29,33}.

The relationship between EV-D68 genetic variations and virulence or pathogenicity is an active area of research. Some studies have suggested that certain genotypes or specific amino acid changes in viral proteins, such as the VP1 capsid protein, may be associated with increased disease severity or the ability to cause respiratory complications. However, more research is needed to fully understand the molecular determinants of EV-D68 pathogenicity^{6,7,19,27,30}.

Phylogenetic and phylogeographic analysis of Enterovirus D68

Data from nextstrain (<https://nextstrain.org/enterovirus/d68/genome>) with 976 whole genome sequences (WGS) of EV-D68 collected between September 1977 to September 2022 showed substitution rates of 4.06×10^{-3} (Highest Posterior Density [HPD 95%]: 3.88×10^{-3} to 4.35×10^{-3}) nucleotides per site per year (s/s/y) (Figure 1).

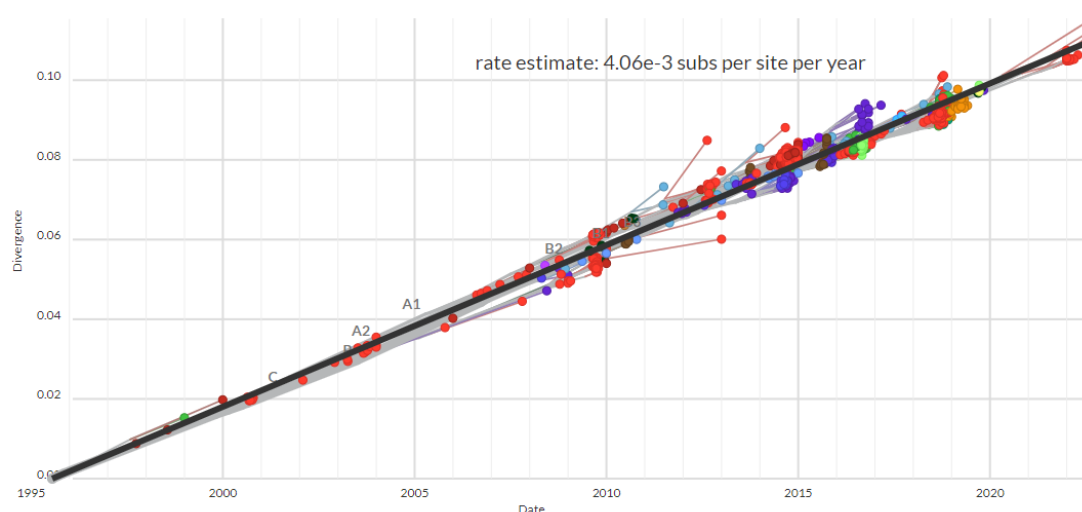


Figure 1: Root-to-tip regression of 976 complete genomes of enterovirus D68 (sequences collected between September 1977 and September 2022) by countries obtained from GISAID.

Figure 2 shows the phylogenetic tree of EV-D68 WGS in the world by clades (A1, A2, B, B1, B2, B3, and C). Analysis of recent evolution of EV-D68 shown that the time to the most recent common ancestor (tMRCA) of A1 was 2005-04-17 (Highest Posterior Density [HPD95%]: 2005-01-09; 2005-09-01) in the USA, A2 was 2003-12-23 (HPD95%: 2003-07-14;

2004-06-06) in China, B was 2003-07-06 (HPD95%: 2003-01-21; 2003-09-28) in China, B1 was 2010-03-21 (HPD95%: 2009-12-20; 2010-05-19) in Vietnam, B2 was 2006-11-25 (HPD95%: 2006-09-07; 2007-03-19) in Vietnam, B3 was 2011-01-15 (HPD95%: 2010-09-07; 2011-03-30) in China, and C was 2000-06-27 (HPD95%: 2000-05-21; 2000-07-24) in the USA.

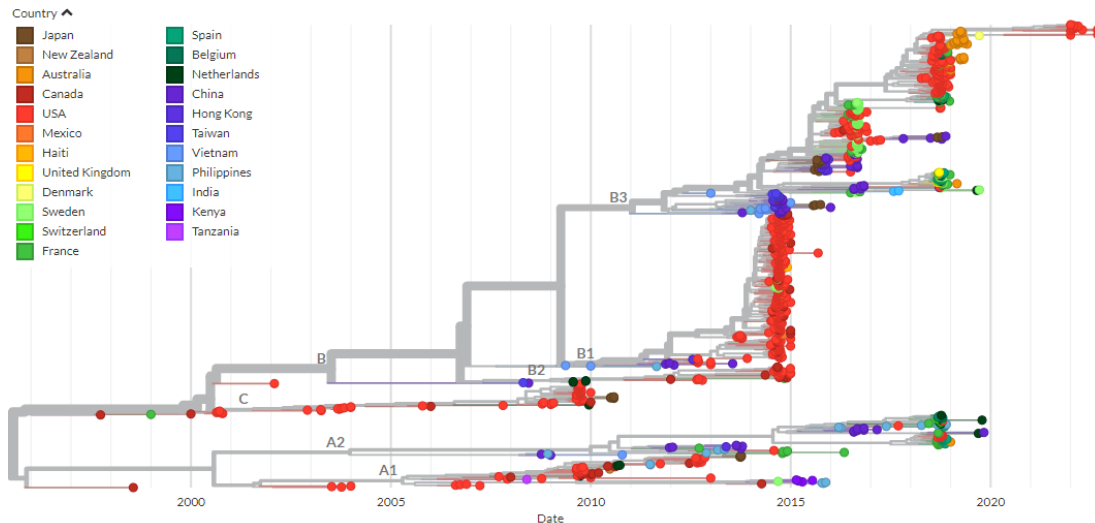


Figure 2: Time-scaled maximum clade credibility tree from the evolutionary reconstruction by Bayesian analysis of enterovirus D68 (976 complete genome sequences collected between September 1977 and September 2022) by countries obtained from GISAID.

The geographic distribution of EV-D68 clades that circulated in the world is shown in Figures 3 and 4. The molecular origin of the EV-D68 was in Canada in 1995 (Bayes Factor [BF]=38), later it was disseminated in France in 1997 (BF=23), the USA in 1999 (BF=30), Taiwan in 2008 (BF=25), Tanzania in 2008 (BF=15), China in 2008 (BF=31), Philippines in 2008 (BF=10), Vietnam in 2009 (BF=15), the Netherlands in 2009

(BF=11), New Zealand in 2010 (BF=10), Japan in 2010 (BF=12), Hong Kong in 2012 (BF=17), Mexico in 2014 (BF=12), Kenya in 2015 (BF=8), Sweden in 2016 (BF=12), India in 2017 (BF=10), Switzerland in 2018 (BF=9), Spain in 2018 (BF=10), Belgium in 2018 (BF=8), Australia in 2018 (BF=9), and Denmark in 2019 (BF=10). Recently, in 2022 this virus circulated in the USA.

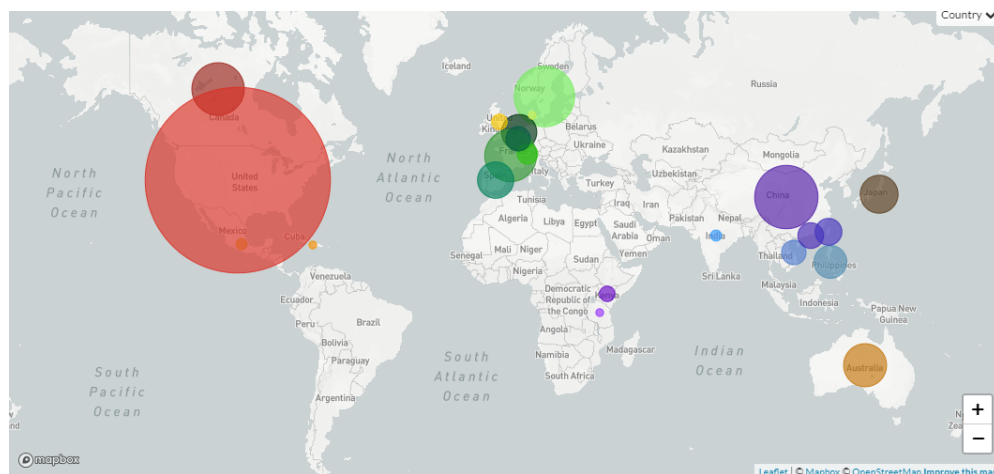


Figure 3: Enterovirus D68 dissemination in worldwide.

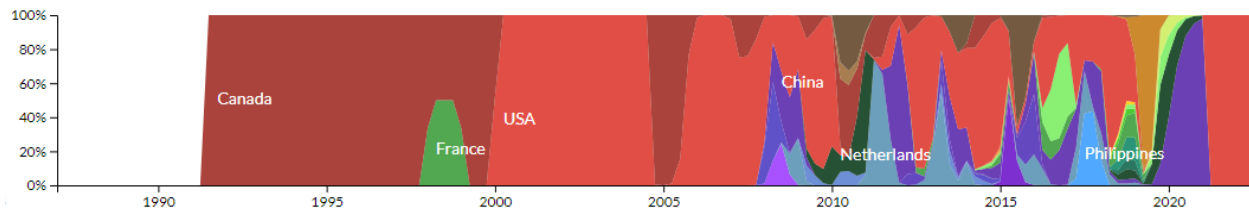


Figure 4: Frequencies of enterovirus D68 colored by country.

Enterovirus D68 immunology

Upon infection with EV-D68, the innate immune system is activated as the first line of defense. Innate immune cells, such as macrophages and dendritic cells, recognize viral components through pattern recognition receptors (PRRs) and initiate a cascade of immune responses. This includes the release of cytokines and chemokines that recruit and activate other immune cells^{6,34-36}.

The adaptive immune response plays a crucial role in clearing EV-D68 infection and providing long-term immunity. T lymphocytes, specifically CD4+ and CD8+ T cells, recognize viral antigens presented by infected cells. CD4+ T cells help coordinate the immune response by activating other immune cells, while CD8+ T cells directly target and kill infected cells^{6,34-36}.

B lymphocytes produce antibodies in response to EV-D68 infection. Neutralizing antibodies, which can bind to the virus and prevent its entry into host cells, are particularly important for limiting viral spread. The production of specific antibodies against viral proteins, such as the capsid protein VP1, contributes to the clearance of the virus^{35,36}.

Following EV-D68 infection, the immune system develops memory cells that provide long-term protection against reinfection. Memory B cells and memory T cells retain information about the virus, allowing for a faster and more effective immune response upon subsequent encounters with EV-D68^{6,34-37}.

Host Factors: Individual variations in the immune response can influence susceptibility to EV-D68 and the severity of the disease. Factors such as age, pre-existing immunity, and underlying health conditions, including respiratory disorders like asthma, can impact the immune response and contribute to differences in disease outcomes^{6,10,34-38}.

In some cases, EV-D68 infection has been associated with immune-mediated complications. It has been hypothesized that an overly robust or dysregulated immune response, including excessive production of pro-inflammatory cytokines, may contribute to respiratory symptoms and severe disease manifestations in susceptible individuals.^{6,34-36,38} Efforts are underway to develop vaccines against EV-D68. Vaccine candidates aim to stimulate both antibody and T-cell responses to provide protective immunity. However, vaccine development for EV-D68

is challenging due to the genetic diversity of the virus and the need to target multiple genotypes.

Understanding the immunology of EV-D68 is essential for developing effective preventive measures, treatment options, and vaccines. Ongoing research continues to shed light on the immune response to EV-D68 infection and how it can be harnessed to mitigate the impact of the virus^{6,10,34-36,38}.

Enterovirus D68 prevention

Preventing the spread of EV-D68 involves implementing a combination of personal hygiene practices and public health measures. Here are some key prevention strategies:

Hand Hygiene: Regularly washing hands with soap and water for at least 20 seconds, especially before eating, after using the restroom, and after coughing or sneezing. If soap and water are not available, use an alcohol-based hand sanitizer that contains at least 60% alcohol¹⁻⁵.

Respiratory Etiquette: Cover mouth and nose with a tissue or your elbow when coughing or sneezing. Dispose of used tissues properly and immediately wash hands afterward. If a tissue is not available, cough or sneeze into elbow rather than your hands^{4,5}.

Avoid Close Contact: Limit close contact with individuals who are sick, particularly those displaying respiratory symptoms. Stay at least 6 feet away from people who are coughing, sneezing, or showing signs of illness⁵.

Clean and Disinfect: Regularly clean and disinfect frequently-touched surfaces, such as doorknobs, light switches, countertops, and electronic devices. Use EPA-approved disinfectants that are effective against viruses⁵.

Stay Home When Sick: If child are experiencing symptoms of respiratory illness, such as coughing, sneezing, or fever, it is important to stay home from work, school, or public places to prevent the spread of EV-D68 and other viruses^{1-5,39}.

Maintain Respiratory Health: Take steps to maintain good respiratory health, especially if you or your child have asthma or other respiratory conditions. Follow your healthcare provider's instructions for managing your condition and ensure medications are taken as prescribed⁵.

Stay Informed: Stay updated with information and guidelines provided by local health authorities, such as the Centers for Disease Control and Prevention (CDC) or the World Health Organization (WHO). They can guide prevention strategies and any specific recommendations during outbreaks or high-risk periods⁵.

Enterovirus D68 diagnosis

A healthcare provider will assess the patient's medical history, symptoms, and physical examination findings. EV-D68 primarily causes respiratory illness, so symptoms like coughing, sneezing, runny nose, wheezing, and difficulty breathing may raise suspicion of EV-D68 infection. However, these symptoms can also be caused by other respiratory viruses, so laboratory confirmation is essential^{10,40-43}.

The preferred method for EV-D68 diagnosis is molecular detection through reverse transcription-polymerase chain reaction (RT-PCR) assays. These tests detect the presence of EV-D68 RNA in respiratory specimens, such as nasal or throat swabs or nasopharyngeal aspirates. RT-PCR provides rapid and specific identification of the virus⁸. Serological testing, specifically testing for the presence of EV-D68-specific antibodies in blood samples, can be performed to determine past exposure or recent infection. However, serological testing is less commonly used for acute diagnosis due to the need for paired acute and convalescent samples²⁷.

Knowledge of EV-D68 activity in the community or region can assist in the diagnosis. If EV-D68 outbreaks are occurring or there is a high prevalence of cases in the area, it increases the likelihood of EV-D68 infection in symptomatic individuals^{10,40-44}. It is important to consult with a healthcare professional for accurate diagnosis and guidance on appropriate testing. EV-D68 shares symptoms with other respiratory viruses, and laboratory confirmation is necessary to distinguish it from other similar illnesses. Additionally, healthcare providers may consider testing for other respiratory pathogens to rule out co-infections or alternative diagnoses^{10,43,45}.

Prompt diagnosis of EV-D68 is crucial for appropriate patient management, implementation of infection control measures, and surveillance purposes. Healthcare providers should follow local guidelines and consult with public health authorities for specific recommendations on EV-D68 diagnosis and reporting^{10,40,43}.

Enterovirus D68 treatment

Currently, there is no specific antiviral treatment or vaccine available for EV-D68. Treatment for EV-D68 is primarily supportive and focuses on relieving symptoms and managing complications. Here are some key aspects of EV-D68 treatment:

Symptom Relief: Over-the-counter pain relievers and fever reducers, such as acetaminophen or ibuprofen, can help alleviate fever, muscle aches, and discomfort. It is important to follow the recommended dosage

instructions and consult a healthcare provider if there are any concerns or if the symptoms worsen^{10,45,46}.

Respiratory Support: Individuals with severe respiratory symptoms, such as wheezing or difficulty breathing, may require medical intervention. In such cases, healthcare providers may administer bronchodilators to help open the airways or provide supplemental oxygen therapy to support breathing^{10,46}.

Fluids and Rest: It is important to stay well-hydrated by drinking plenty of fluids, especially if there is fever or respiratory symptoms. Getting adequate rest is also crucial for the body's recovery and immune response.

Individuals with pre-existing asthma or other respiratory conditions should ensure that their condition is well-managed. Following the prescribed asthma management plan, using prescribed inhalers or medications as directed, and monitoring symptoms are important to prevent exacerbations and complications. In severe cases, particularly when respiratory distress is present, hospitalization may be necessary. Hospital-based care can provide intensive monitoring, oxygen therapy, and other interventions to support the patient's respiratory function and overall well-being^{10,45,46}.

CONCLUSION

EV-D68 is a respiratory virus that primarily affects children and can cause a range of respiratory symptoms, from mild cold-like symptoms to severe respiratory illness. The epidemiology of EV-D68 is characterized by sporadic outbreaks, often occurring during late summer and fall. Enhanced surveillance helps monitor its activity and track circulating strains.

Literature reports that EV-D68 was discovered in 1962 after being isolated from respiratory specimens of children with pneumonia in the USA. The molecular evolution of EV-D68 involves genetic diversity, with multiple clades identified worldwide. EV-D68 spread in North America to Europe, Asia, Oceania, Latin America, and Africa. Recently, in 2022 this virus circulated mainly in the USA.

The immune response to EV-D68 involves both innate and adaptive immunity, including the production of neutralizing antibodies and the activation of T cells. Individual factors, such as age and underlying health conditions, can influence the immune response and disease outcomes.

Preventing the spread of EV-D68 involves practicing good hand hygiene, respiratory etiquette, and avoiding close contact with sick individuals. Regular cleaning and disinfection of surfaces are important preventive measures. Staying informed and following guidelines from health authorities is crucial in preventing and managing EV-D68 infections. Currently, there is no specific antiviral treatment or vaccine available for EV-D68. Treatment focuses on supportive care, including symptom relief, respiratory support if necessary, adequate hydration, rest, and management of underlying health conditions.

Also, it is important to note that the molecular evolution of EV-D68 is an ongoing field of study, and new findings may emerge as research progresses. Continued surveillance and genetic analysis are essential for monitoring the virus's evolutionary changes and informing public health interventions.

Authorship

Jonas Wolf designed the study. Jonas Wolf wrote the first draft of the manuscript and contributed to the literature review and discussion of the results.

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Conflicts of interest

The author declare no conflicts of interest.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study

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