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The Emergence of Coxsackievirus A16 Associated with HFMD Colloquially Termed 'Tomato Flu' in India 2022: A Detailed Virological Inspection

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Abstract

As the world battles the COVID-19 pandemic and grapples with a concerning spike in monkeypox cases, India has encountered another viral illness dubbed "Tomato Flu." Tomato Flu is a viral disease caused by coxsackievirus A16, which is a highly infectious illness affecting children under 10 years old. It is also known as hand, foot, and mouth disease (HFMD), and is caused by Enterovirus A71, CV-A16, CVA6, and Echoviruses. The epidemiological attributes of this disease have been documented, and the replication process of CV-A16 is closely related to that of enterovirus infection. The main avenue of viral transmission is through close interaction with infected children. Symptoms include blisters beneath the palms and feet, rashes throughout the body, fever, dehydration, and joint swelling. Prevention is crucial, including hydration, avoidance of touching blisters, proper isolation, hygiene maintenance, and frequent washing or sanitizing. General treatment can be applied to both Tomato Flu and HFMD manifestations. Currently, there is no available tomato flu vaccine; however, the CV-A16 vaccine from Lactococcus lactis has been developed as an oral vaccine to prevent CV-A16. An inactivated vaccine was also produced using the CVA16-393 strain. However, further investigation is required to ascertain its applicability in this context.

Keywords: Coxsackievirus A16, Tomato flu, Enterovirus 71, HFMD, Epidemiology, *Lactococcus lactis*, Oral vaccine, Inactivated vaccine

INTRODUCTION

Communicable diseases have been advancing in the modern era. The first coronavirus epidemic happened in China exactly five years ago, in 2019. This situation escalated into an unprecedented global pandemic in the twenty-first century [1]. India currently deals with the fourth wave of the COVID-19 outbreak, with a significant rise in monkeypox cases [2].

Another new contagious viral disease first emerged in India's Kollam district of Kerala in May 2022. This disease exhibits typical flu manifestations, including pyrexia, tiredness, and myalgia. It was distinguished by the appearance of red, irritating blisters that expanded to the size of tomatoes, giving it the moniker "Tomato Flu." This infection quickly propagates among young children, similar to conventional hand-foot-and-mouth disease (HFMD). The coxsackievirus A16 (CV-A16) has been confirmed to be responsible for the outbreak of tomato flu. The term "tomato flu" has lately been criticized as misleading, and the clinical characteristics of this illness are described as an atypical manifestation of HFMD [3]. Although no deaths have been reported at present, tomato flu

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disease in India represents a concern for the health of children in India, its surrounding nations, and throughout the world [4]. The incidence of the tomato flu has a huge negative impact on India's growing child population. The virus has low virulence, frightening rates of propagation, and worrying levels of inactivity, making it unlikely that it will cause a pandemic. However, if sufficient biosecurity precautions are not implemented, it might pose a serious public health risk to the kids [5].

A relatively common viral infection in children, hand, foot, and mouth disease (HFMD), was first identified in 1948. The two main viruses that cause HFMD are human enterovirus 71 (EV-A71) and coxsackievirus A16 (CV-A16). HFMD has not attracted much attention for a very long period since the infection was thought to be a moderate viral infection linked with self-limiting clinical symptoms that disappeared after 5–7 days [6]. However, a substantial percentage of patients experience catastrophic cardiovascular or neurological disorders. Furthermore, current investigations have demonstrated that patients who have healed substantially may experience serious neurological sequelae [7-9]. As a result, HFMD has become a serious public health issue both inside and outside the Asia-Pacific region. A fresh focus has been placed on HFMD outbreaks since the discovery of the tomato flu, an enterovirus-caused illness in India [10]. In addition to coxsackieviruses, enteroviruses also comprise the poliovirus and echovirus subgroups. It is estimated to have 1 billion or more enteroviral infections per year. Over 50% of enteroviral diseases in the United States each year are caused by coxsackievirus A6, coxsackievirus A9, coxsackievirus B4, echovirus 6, echovirus 11, echovirus 18, echovirus 30, and enterovirus D69 [11].

EPIDEMIOLOGY OF TOMATO FLU IN INDIA

In May 2022, a 13-month-old girl and her 5-year-old brother developed rashes on their hands and legs. On family holidays, they visited Kerala in several locations. While they had been there, the local Kerala media were reporting on an unknown disease that children were suffering from that was called the "tomato flu." The kids were covered in sores when they arrived back in the UK, with the girl standing out the most (Figure 1). There were no fevers or systemic signs among the kids. The kids had examinations for enterovirus (EV) presence. Because of the fleshy and vesicular nature of the rashes, the girl's specimens were further checked for monkeypox at Porton Down, Salisbury, UK, a national reference laboratory. The girl's monkeypox PCR test was negative, but the EV PCR results for both kids were positive. It was discovered that Coxsackie A16 was the result of EV typing via sequencing at a different national reference laboratory (UKHSA-Colindale, London, UK) [12].

The tomato flu was first identified in the Kollam district of Kerala on May 6, 2022. Later, the virus spread to Neduvathur, Aryankavu, and Anchal in Kerala. In Kerala, 82 cases of the disease were identified by the end of July 2022, according to Lancet Respiratory Medicine [10]. In 2007, the virus

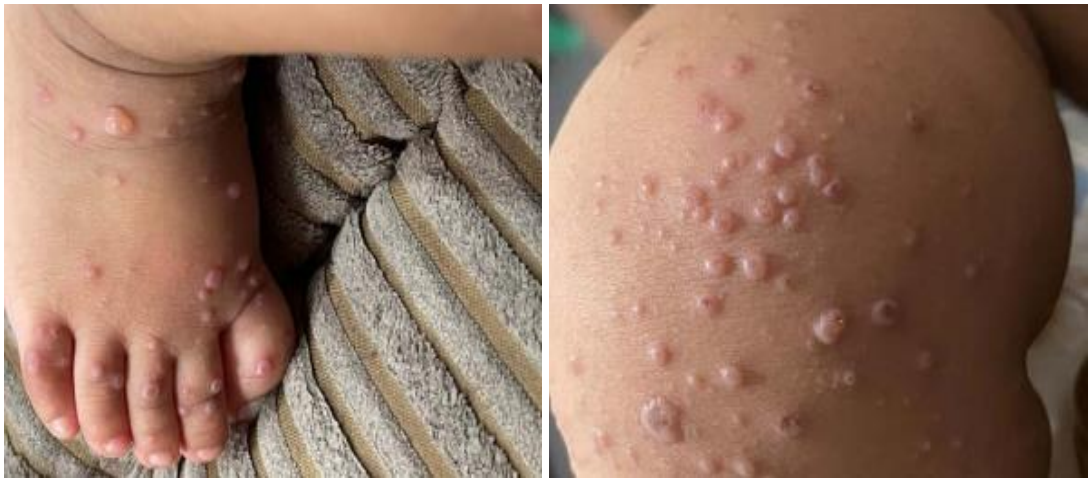

Figure 1. The lacerations were evident on the limbs, palmar, and plantar sides of a 13-month-old girl (credit: Julian W. Tang [12]).

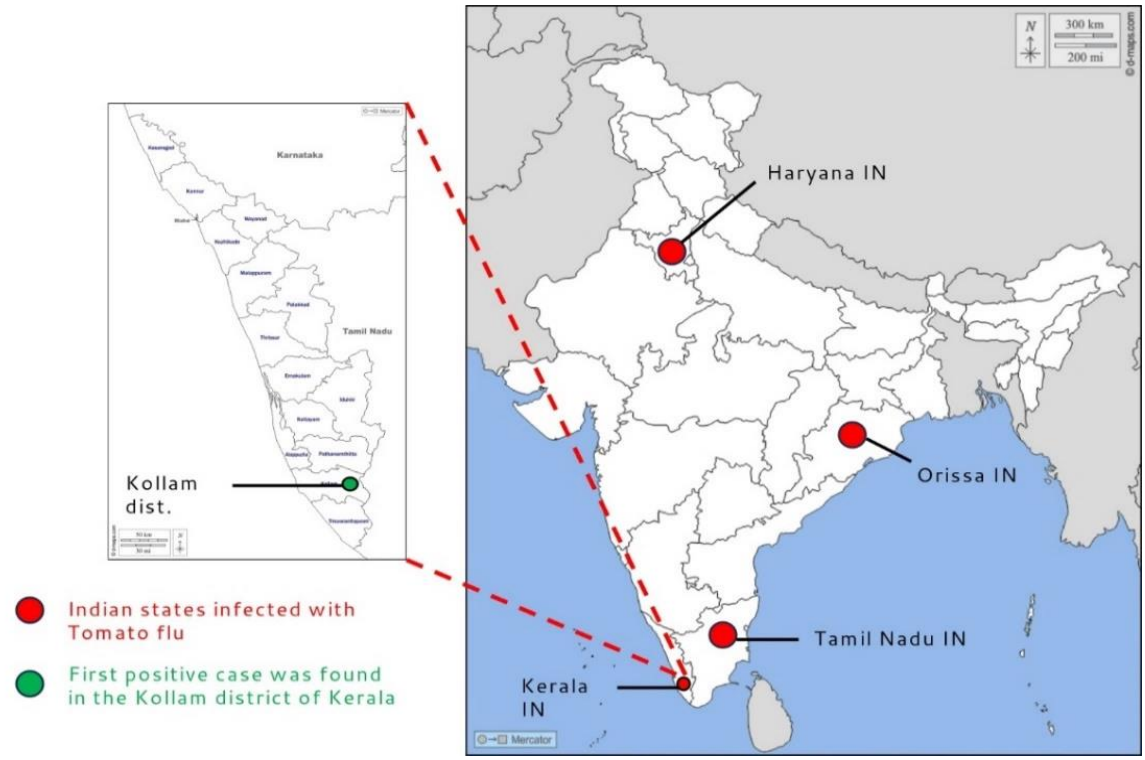

Figure 2. Map highlighting the Indian states where most tomato flu cases were found.

first attacked Kerala. The tomato flu has reportedly expanded to the states of Kerala, Odisha, and Haryana, as reported by the Tamil Nadu government's healthcare department [13]. Additionally, according to the Regional Medical Research Centre in Bhubaneswar, the sickness affected 26 children in Odisha, ages 1 to 9 [14]. As a result, several hundred children in southern Indian cities have been diagnosed with tomato flu, also known as Hand, Foot, and Mouth Disease (HFMD) (Figure 2) [15]. The majority of these children are under the age of 10. Similar to HFMD, this viral disease has resulted in the appearance of blisters and rashes [16], [17]. The disease got its name from the enormous red blisters that resembled tomatoes that appeared on the skin of persons who had it. The coxsackievirus that causes HFMD is sometimes referred to as tomato flu, an inaccurate term that gained widespread recognition [18].

Coxsackie Virus A16 (CV-A16) had been previously associated with fatal HFMD cases in mainland China, Taiwan, Japan, France, and the United States. In Shenyang, China, 92 HFMD cases with neurological impairments were documented in 2010. Nineteen instances of CV-A16 infection were identified, of which two resulted in brainstem encephalitis and one in acute flaccid paralysis [19].

ETIOLOGY OF COXSACKIEVIRUS A16

In 1951, the CV-A16 virus was first isolated in South Africa [20]. It belongs to the *human enterovirus A* (HEV-A) species of the *Enterovirus* genus *Picornaviridae*. The CV-A16 virus has an icosahedral, non-enveloped, tiny (diameter ~30 nm) RNA genome with a single-stranded, positive-sense, polyadenylated tail of about 7.4 kb. An large polyprotein precursor encoded by a single reading frame in the genome is subsequently transformed into structural protein P1, nonstructural proteins P2, and P3. P1 is changed into the viral capsid subunit proteins VP0, VP1, and VP3 by the virus-encoded proteinase. There are other ways to dissect VP0 in order to produce VP2 and VP4. VP1, VP2, and VP3 are found in the capsid's outermost region, while VP4 is found in its innermost region. The majority of the neutralizing epitopes were found on VP1. The untranslated regions (UTR) present at the 5′ and 3′ termini of this virus provide it an edge over its cistron. The internal ribosome entry site (IRES) is present in the 5′ UTR region, which is approximately 740 nucleotides long and comprises sequences that regulate

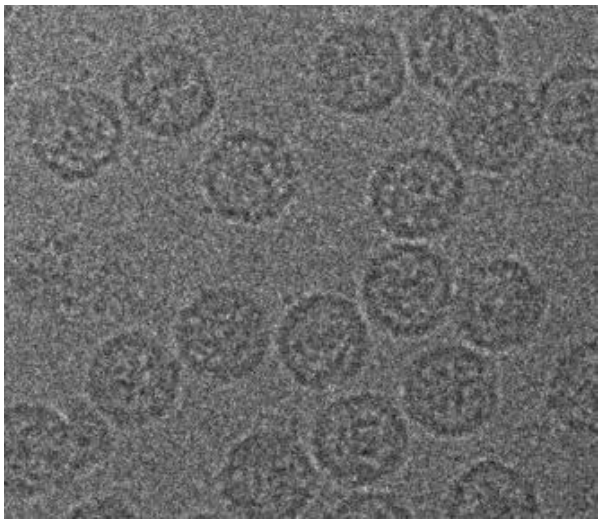

Figure 3. A cryo-electron micrograph of the Coxsackievirus A16 (Image credit: Gong [22]).

genome replication and translation. The polyA tail present in the 3′ UTR region is crucial for viral infectivity [21]. The icosahedral CV-A16 particle has no envelope and is composed of 60 copies of VP1, VP2, and VP3 on the outside and VP4 and their N-terminal extensions on the inside [19].

A cryo-electron micrograph of a CV-A16 virus-like particle was obtained using an FEI Titan Krios electron microscope (Figure 3) [22].

Mode of Replication

To date, humans are CV-A16's only known natural host. Most animal viruses have host-range specificity and tissue tropism, which are determined by specific cellular receptors [23]. It has been reported that hSCARB2 (human scavenger receptor class B, member 2) is a potential cellular receptor for CV-A16. In a study on the maturation of a murine model of CV-A16 infection, the skeletal muscle emerged as the predominant site of early viral replication, whereas the brain was identified as the site of transmission and replication later in the infection cycle. Another study reported that CV-A16 exhibited tropism to lung and brain tissues instead of to muscle tissues in nasally infected hSCARB2 transgenic mice [24]. Initially, the viral genome functions as a template for translation, leading to the generation of a viral polyprotein, and then as a template for replication when the virus enters a permissive host cell. Internal ribosome entry sites (IRESs) within the 5' UTR play a crucial role in translation initiation, whereas the 5' terminal cloverleaf structure is significantly involved in viral replication. The 5'-UTR is strategically essential for both these functions. However, an experiment in a neonatal paradigm demonstrated that the 5'-UTR of CV-A16 is essential for viral replication and pathogenicity. The specific mechanism of CV-A16 replication remains unclear [25].

Much of our knowledge about the Coxsackievirus RNA replication process is closely related to enterovirus infection. Coxsackievirus belongs to the *Picornaviridae* family and is classified within the genus *Enterovirus* [26]. Endocytosis begins when an enterovirus binds to a receptor. This receptor interacts with the five-fold axis of symmetry and is surrounded by a depression in the capsid known as the canyon. The virus is subsequently internalized and releases its RNA into the cytoplasm, which acts as a template for translation to produce polyproteins. Viral proteases $2A^{pro}$ and $3C^{pro}$ catalyze the cleavage of polyproteins, resulting in the release of structural and non-structural viral proteins, along with specific stable precursors. Viral proteases also break down cellular targets to enhance favorable conditions for viral development and block innate antiviral responses. Replication of the viral genome is mediated by the non-structural proteins P2 and P3. The RNA-dependent RNA polymerase $3D^{pol}$ of the virus uses the secondary RNA structure referred to as the cis-acting replication element (Cre) in the

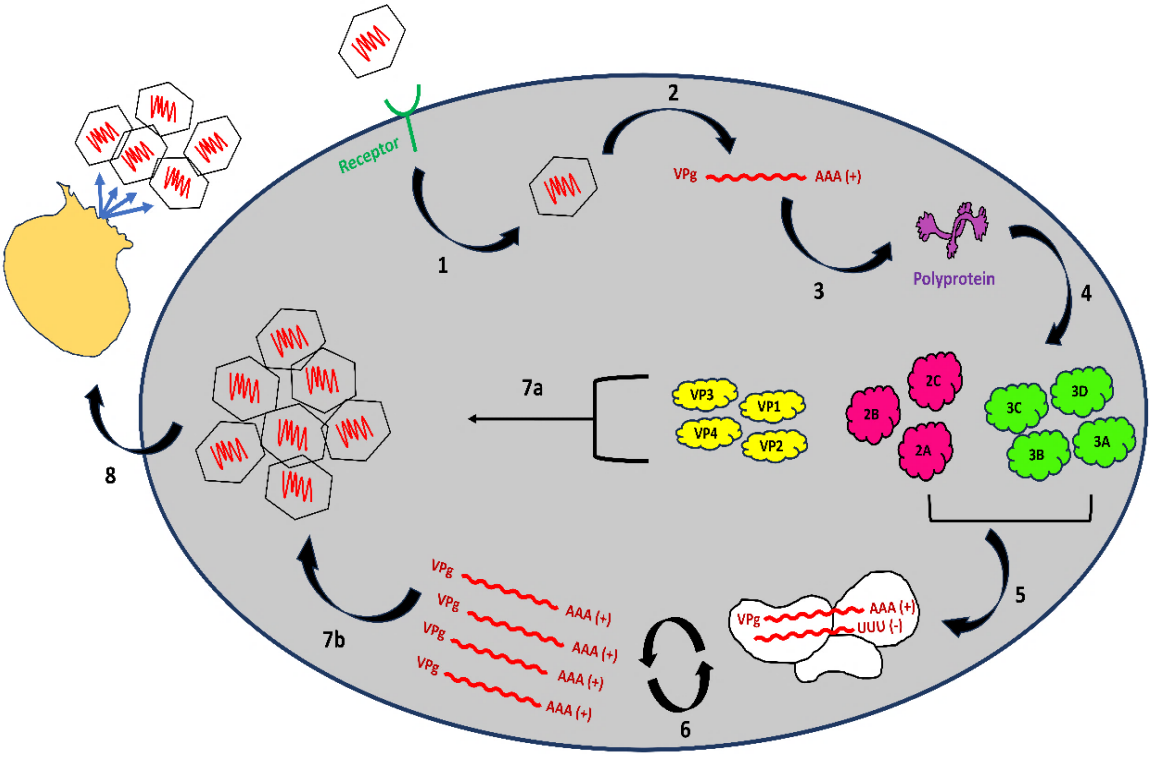

Figure 4. Illustration of the replication mode of enterovirus (credit: Ismail [27]). (1) Attachment of the receptor to the viral canyon; (2) Release of viral RNA into the cytoplasm; (3) Template for translation and generation of polyproteins; (4) Release of structural and nonstructural viral proteins by proteolytic processing of polyproteins. Subsequently, these proteins were generated; (5) Viral RNA replication is mediated by viral replication proteins; (6) As a result, a negative-stranded intermediate is generated, which is then used as a template for the synthesis of positive-stranded RNA molecules, which can then undergo another cycle of translation; (7) Formation of the capsid by the capsid proteins (7a) into which the positive strand RNA is packed (7b) to make up the infectious viral particles; (8) Cell lysis results in the release of new virus-like particles [27].

viral genome as a template to initiate RNA genome replication by uridylating the protein primer VPg. A negative-stranded intermediate generated by $3D^{pol}$ elongation of VPg was subsequently utilized as a template, enabling the synthesis of positive-stranded RNA molecules. Positive-stranded RNA molecules have two options: they may either start a new translation and replication or be stuffed into capsids to become infectious viruses. As a result of cell lysis and other non-lytic processes, these new viral particles are released (Figure 4) [27].

Pathogenicity of EV-A71 and CV-A16

The most recent wave of tomato flu, often self-limiting and less lethal, was found to be associated with CV-A16. The emergence of the disease is considered more severe in HFMD with EV-A71. In an investigation comparing 177 EV-A71 and 64 CV-A16 patients during Taiwan's enterovirus outbreak, aseptic meningitis only occurred in 6.3% of CV-A16 infections [28]. However, encephalomyelitis, encephalitis, polio-like syndrome, aseptic meningitis, and fatal pulmonary edema were observed in 32% of the EVA71 cases. It has been determined that the non-structural (NS) proteins of CV-A16, essential for translation, replication, and host cell subversion, are produced by cleaving P2 and P3. Cellular endocytosis and uncoating of the genetic material are significantly facilitated by capsid proteins by cleavage of P1 [29]. Coxsackie viruses relate to a wide range of diseases. The typical symptoms of CV-A16 types A and B include aseptic meningitis, fever rashes, and non-specific upper respiratory tract

infections. It is well known that CV-A attacks the skin and mucous membranes in particular. The pathogenesis of these viruses is regulated by particular interactions between the virus and receptor, defining the initial viral infection's source. It further facilitates the virus's spread to additional organs when the infection progresses into the post-viremic stage [30]. Both EV-A71 and CV-A16 make use of human scavenger receptor class B member 2 (hSCARB2), which is present in a variety of types, including neuroglial cells [31]. One essential enzyme for single-stranded RNA replication in the picornavirus genomes of both CV-A16 and EV-A71 is RNA-dependent RNA polymerase (RdRp). These RNA viruses have an excessive quantity of mutation, which prevents RdRp from proofreading. The emergence of novel proteins owing to numerous mutations, as demonstrated in several studies, is pivotal for the adaptation of viral replication, survival, and virulence in EV-A71.

Another example is the replacement of methionine (M) with lysine (K) at position 149 in the VP2 amino acid ($VP2^{K149M}$), which has been linked to an increase in RNA accumulation, viral cytotoxicity, and uncoating in mouse neural cells, in addition to an enhancement in mouse lethality in vivo. Additionally, $VP1^{145Q}$ has been identified as an important factor for enhanced infectivity in human airways. It was also discovered that the $VP1^{D31G}$ mutation increased the rapidity at which EV-A71 entered neuroblastoma, increased the viral growth rate in human neural cells, and resulted in a higher percentage of mortality from the virus than in HFMD [32].

The primary attachment residues to its receptors, P-selectin glycoprotein ligand-1 (PSGL-1) and scavenger receptor B2 (SCARB2), are found in the structural protein VP1 of EV-A71. Furthermore, EV-A71's 3C protein inhibits the cytosolic retinoic acid-inducible gene I (RIG-I). Effectively stopping the onset of antiviral immunity in the human body, EV-A71 decreased the expression of IFN-β, IFN-stimulated gene 54 (ISG54), ISG56, and tumor necrosis factor alpha in infected cells [33]. Through recombination, the most common genotype of CV-A16 regularly aids in the creation of novel viral variants. A novel version of CV-A16 may have produced the recent tomato flu outbreak and its unique lesions, since RNA viruses are more prone to mutate since they lack proofreading [34].

EPIDEMIOLOGY OF HAND, FOOT, AND MOUTH DISEASE

HFMD was first reported in New Zealand in 1957. The next year, in 1958, Coxsackievirus A16 was first identified in Canada. The name HFMD originates from common maculopapular or vesicular lesions that affect the hands, feet, and oral mucosa [35]. It mostly affects children under the age of 5 [36]. The study of HFMD epidemiology focuses on regions where the illness is widespread, and there have been significant severe cases. A final example is the World Health Organization () Western Pacific Region [37]. Since the 20th century, HFMD cases have been documented across Asia and are present as both local outbreaks and sporadic incidences. Since 1997, epidemic-scale HFMD outbreaks have been reported in an array of Asian countries, including China, Malaysia, Taiwan, and Singapore [38]. In 2008, an HFMD virological monitoring program was established by the Ministry of Health of China, following multiple significant outbreaks of HFMD in 2007 and early 2008. HFMD is categorized as a class-C notifiable infectious disease. The primary manifestations of HFMD include fever and common vesicular blisters in the hands, feet, buttocks, or mouth. Although the majority of HFMD patients experience minor, self-limiting symptoms, they are occasionally susceptible to more severe conditions, including acute flaccid paralysis, myocarditis, encephalitis, and encephalomyelitis [36]. Additionally, it has been reported that the tomato flu is infectious and spreads through intimate contact [19].

Global reports of HFMD epidemics during the last few decades have mostly implicated Enterovirus A71 (EV-A71), CV-A16, CVA6, and echovirus (Echo) [39]. Enterovirus A71 (EV-A71) and coxsackievirus A16 (CV-A16) are responsible for the majority of HFMD cases [40]. A countrywide surveillance program for 2022 instances of HFMD indicates that the bacteria responsible are changing. After the inactivated vaccine was made available and actively promoted in China in 2016, the number of EV-A71-related cases of HFMD precipitously decreased [41]. On the other hand, whereas the amount of CV-A16 has steadily increased, the fraction of other EVs in the pathogen spectrum has significantly

changed [42]. According to the statistics, 90% of EV-A71-induced cases of HFMD can be prevented by the monovalent EV-A71 inactivated vaccine. However, it does not offer cross-protection against infection with CV-A16 and other EVs, which contributes to the HFMD cases that are consistent with CV-A16 infection. Since HFMD was recognized as a reportable infectious illness in 2008, there have been around 24.57 million cases documented in China as of December 2022. According to the statistical data obtained from the National Health Commission of the People's Republic of China, 3698 deaths have resulted from these cases [36].

In India, significant HFMD outbreaks were documented in Kerala in 2003 and West Bengal in 2007. While EV-71 was the primary cause of the 2003 endemic in Kerala, the causal agents in the country's eastern and southern states between 2010 and 2017 were CV-A6 and CV-A16. In 2022, CV-A6 and CV-A16 strains were the major EV strains that caused HFMD in Karnataka and in Assam, where a hand-foot-mouth disease caused by CV-A16 affected thousands of children [38, 43].

TRANSMISSION AND SYMPTOMS OF TOMATO FLU

The initial research demonstrated that tomato flu, actually HFMD, is a highly infectious illness spreading through intimate contact, rendering young children under five years old at risk. The most common ways that infants get sick are through their diapers, touching dirty surfaces, or putting contaminated things in their mouth (Figure 5) [44].

Tomato flu is a viral disease caused by CV-A16. The main manifestation of tomato flu is blisters shaped like tomatoes that develop on different body parts, and that is the reason from where the name tomato flu comes from. Initially, the blisters appear as little red pimples that, as they develop, look like tomatoes. Fever, rashes, and arthritis in the joints are typical manifestations of other illnesses caused by viruses, and they also appear among kids suffering from tomato flu. Itching may also result from rashes on the skin. The symptoms are similar to those of other infections caused by viruses, such as typical influenza-like symptoms including tiredness, nausea, vomiting, diarrhea, fever, dehydration, joint swelling, and body pains. HFMD is characterized by fever, oral ulcers, and skin blisters as its primary symptoms. Fatigue, low appetite, slight fever, and throat irritation are common symptoms. Little red patches that develop into blisters and eventually ulcers develop one or two days after the fever starts. Typically, the sores were observed on the tongue, interior of the cheeks, gums, palms, and soles. After several days, the symptoms and indications of the tomato flu go away, making it a self-limiting infectious disease [45].

PREVENTIVE MEASURES

After the unexpected wave of the CV-A16 viral disease, prevention is crucial. The effects of an epidemic may be readily contained if everyone properly adheres to preventative measures. However, there is uncertainty over the level of observance with preventative measures since children under the age of five are often affected by the tomato flu [46]. Listed below are a few measures to avoid spreading the tomato flu.

1. *Proper hydration:* It is important to consume additional boiling water, juices, and other liquids to keep the body well hydrated.
2. *By avoidance of touching blisters:* Patients may prevent the spread of the illness by avoiding touching blisters, practicing proper personal cleanliness, and avoiding close proximity to suspicious situations. To avoid the tomato flu's chronic symptoms, maintain a proper circadian rhythm.
3. *Isolation:* Tomato flu is highly contagious. Therefore, it is crucial to properly isolate any cases of the tomato flu virus that have been confirmed or suspected as well as to implement additional protective measures in order to limit the virus's spread. To prevent the virus from spreading to other children or adults, isolation should be used for five to seven days after symptoms start to show.
4. *Hygiene and sanitation:* Maintaining good hygiene, cleaning the environment, and forbidding a sick child from sharing toys, clothing, food, or other objects with other healthy kids are the greatest ways to avoid infection [47].

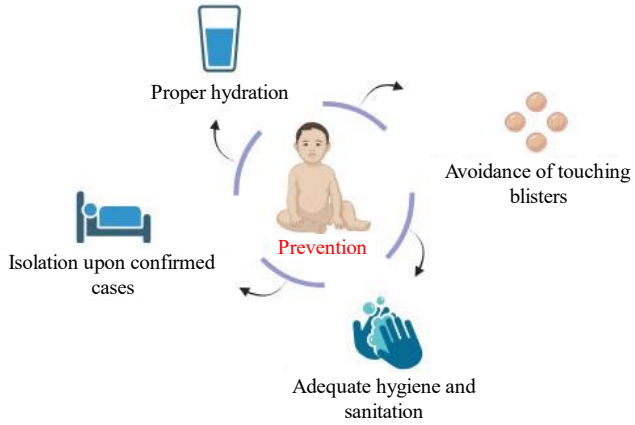

Figure 5. A schematic diagram of the prevention of the spreading of the tomato flu.

TREATMENT

There is currently no appropriate vaccine for tomato flu as well as HFMD, which is associated with CV-A16 [36]. A general treatment regimen is applied to typical scenarios.

1. *Antiviral treatment:* Treatment with IFN-α and ribavirin has demonstrated some favorable effects in the antiviral therapy of HFMD.
2. *Intravenous immunoglobulin (IVIG):* IVIG treatment may significantly inhibit the disease's development and decrease mortality in the early stages of HFMD. The combination of conventional treatment and IVIG resulted in a quicker recovery from fever, rash regression, and clinical cure as compared to standard therapy administered alone.
3. *Respiratory assistance:* The most effective way to increase the body's oxygen supply is through mechanical ventilation.
4. *Cardiovascular assistance:* In the process of treatment, many inotropes, including epinephrine, milrinone, dobutamine, and dopamine, have been used to sustain heart function. If none of the medications work, levosimendan or vasopressin may be an alternate.
5. *Intracranial pressure regulation:* When combined with hypertonic saline or diuretics, mannitol is often used to lower elevated intracranial pressure [39].

VACCINES UNDER DEVELOPMENT AND CLINICAL TRIALS

Oral Vaccine

Lactococcus lactis is lactic acid bacteria (LAB). The phenotypic characteristics categorize it as a facultative anaerobic gut bacterium that is gram-positive, spherical, homolactate, and non-sporulating [48]. The body benefits greatly from LAB because they sit firmly on the surface of the gastrointestinal (GI) tract as probiotics. *L. lactis* had been applied to produce the CV-A16 VP1 protein to create an oral CV-A16 vaccination. Compared to other released expression proteins, this protein could be of higher merit. The gut must be continuously stimulated by the lactic acid bacteria that release expression proteins. It is possible that the expression protein concentration is low as a result. On the other hand, until the bacteria are destroyed, foreign proteins are continuously expressed within the cell. But lactic acid bacteria-produced oral vaccinations have two issues, one of which is related to stomach acid. Lactis NZ9000's recT gene overexpression improves resistance to acid stress. CV-A16 VP1 was expressed using the lactic acid bacteria NZ9000. It can tolerate the effects of hydrochloric acid. The second issue is drug-resistant genes in recombination-prone lactic acid bacteria. The primary cause of this issue is the bacterial expression plasmid for lactic acid. Non-resistant plasmids have been employed to express foreign proteins because of technological advancements, but screening positive bacteria is difficult. Protective antibodies against CV-A16 have been developed by an oral CV-A16 vaccination derived from *Lactobacillus*. It could therefore serve as an oral vaccination against CV-A16, although further research, including animal trials, is needed to determine its usefulness in this regard [49].

Inactivated Vaccine

A successful neutralizing antibody response in gerbils has been induced by the CV-A16 vaccination developed from the CVA16-393 strain. In a gerbil model, the CV-A16 vaccination further demonstrated cross-protection against fatal challenges from strains of the B1a and B1b subtypes from different regions of China. The viable strain to consider as a vaccine candidate is CVA16-393, since it shows potential benefits for vaccine development in MRC-5 cells. The effectiveness of multiple types of CV-A16 candidate vaccinations, including the adenoviral vector and virus-like particle vaccines, has been tested in animals before being developed. These vaccinations have elicited strong immunological responses in mice, and the resulting maternal antibodies shielded the young mice against the CV-A16 challenge. However, the inactivated vaccine provides an array of benefits over the virus-like particle and adenoviral vector vaccines, including straightforward research simplification and well-established regulatory requirements. Since the COVID-19 pandemic commenced, mRNA vaccines have been extensively applied to humans. The mRNA vaccines do not need pathogen purification or cell culture; they can be generated more rapidly than inactivated vaccines. Most CV-A16 vaccines that have been found to cause effective protection have been made in Vero cells. But only a few of them have derived from the human diploid cell line KMB17. But no CV-A16 vaccination has yet been developed with MRC-5 cells. The production of vaccines for rabies, MMR, varicella (chickenpox), hepatitis A, shingles, and polio have incorporated the uses of MRC-5 cells, a human diploid cell line. Certain inactivated vaccinations are often less immunogenic than their live equivalents, even though inactivated vaccinations are thought to be more secure and stable than live vaccinations. Currently, several animal models have been produced to evaluate the CV-A16 vaccinations and investigate the pathophysiology of the CV-A16 virus. Furthermore, the CVA16-393 strain has produced a CV-A16 vaccine that effectively protects against cross-protection in gerbils, indicating that this strain might be a standard choice for the development of a CV-A16 vaccine [50].

CONCLUSION

The cases of tomato flu were identified during a period of rising anxiety about the nationwide monkey pox epidemic. This study highlights the epidemiological characteristics of tomato flu, along with HFMD and the main causative agent, CV-A16. Even though the virus was spreading decisively, the government's quick response in terms of treatment and prevention substantially inhibited its spread. The production of vaccines for rabies, MMR, varicella (chickenpox), hepatitis A, shingles, and polio has incorporated the use of MRC-5 cells, a human diploid cell line. There are no vaccines or proper antiviral drugs developed for the prevention of CV-A16-associated tomato flu as well as HFMD. Isolation and quick testing emerged as the most crucial countermeasures to stop the spread of the disease in the absence of vaccinations and diagnostic treatments. Overall, our investigation delivers all the relevant information about CV-A16, which is associated with a recent endemic in India.

REFERENCES

1. Y. C. Wu, C. S. Chen, and Y. J. Chan, "The outbreak of COVID-19: An overview," *Journal of the Chinese Medical Association*, vol. 83, no. 3. Wolters Kluwer Health, pp. 217–220, 2020. doi: 10.1097/JCMA.0000000000000270.
2. "Tomato Flu Outbreak in India: Understanding the disease." Accessed: Sep. 03, 2023. [Online]. Available: https://www.firstpost.com/india/tomato-flu-outbreak-in-india-understanding-the-contagious-disease-11087761.html
3. A. Ismail, A. Saahath, Y. Ismail, M. F. Ismail, Z. Zubair, and K. Subbaram, "'Tomato flu' a new epidemic in India: Virology, epidemiology, and clinical features," *New Microbes and New Infections*, vol. 51. Elsevier Ltd, Jan. 01, 2023. doi: 10.1016/j.nmni.2022.101070.
4. "Children of Today are Leaders of Tomorrow." Accessed: Sep. 03, 2023. [Online]. Available: https://www.unicef.org/tajikistan/press-releases/children-today-are-leaders-tomorrow
5. R. O. Adesola, I. Idris, E. Opuni, and J. N. D'Souza, "Tomato flu 2022 outbreak in India: Issues, challenges, and possible solutions," *Health Science Reports*, vol. 6, no. 4. John Wiley and Sons Inc, Apr. 01, 2023. doi: 10.1002/hsr2.1174.

6. R. C. C. Carmona *et al.*, "Hand, foot, and mouth disease outbreak by Coxsackievirus A6 during COVID-19 pandemic in 2021, São Paulo, Brazil.," *Journal of Clinical Virology*, vol. 154, Sep. 2022, doi: 10.1016/j.jcv.2022.105245.
7. L. Y. Chang *et al.*, "Enterovirus A71 neurologic complications and long-term sequelae," *J Biomed Sci*, vol. 26, no. 1, Aug. 2019, doi: 10.1186/s12929-019-0552-7.
8. G. Gonzalez, M. J. Carr, M. Kobayashi, N. Hanaoka, and T. Fujimoto, "Enterovirus-associated hand-foot and mouth disease and neurological complications in japan and the rest of the world," *Int J Mol Sci*, vol. 20, no. 20, Oct. 2019, doi: 10.3390/ijms20205201.
9. S. H. Hsia, J. J. Lin, O. W. Chan, and T. Y. Lin, "Cardiopulmonary failure in children infected with Enterovirus A71," *Journal of Biomedical Science*, vol. 27, no. 1. BioMed Central Ltd., Apr. 16, 2020. doi: 10.1186/s12929-020-00650-1.
10. V. P. Chavda, K. Patel, and V. Apostolopoulos, "Tomato flu outbreak in India," *The Lancet Respiratory Medicine*, vol. 11, no. 1. Elsevier Ltd, pp. e1–e2, Jan. 01, 2023. doi: 10.1016/S2213-2600(22)00300-9.
11. M. Sandoni *et al.*, "Enteroviral Infections in the First Three Months of Life," *Pathogens*, vol. 11, no. 1. MDPI, Jan. 01, 2022. doi: 10.3390/pathogens11010060.
12. J. W. Tang *et al.*, "Kerala Tomato Flu - A Manifestation of Hand Foot and Mouth Disease," *Pediatric Infectious Disease Journal*, vol. 41, no. 11. Lippincott Williams and Wilkins, pp. E501–E503, Nov. 01, 2022. doi: 10.1097/INF.0000000000003668.
13. "tomato_flu_outbreak_in_india__why_is_it_an.39".
14. "Advisory on Hand Foot and Mouth Disease (HFMD), commonly known as 'Tomato Flu.'"
15. "Over 100 cases of Tomato Flu detected in children under 9 years of age, health ministry issues advisory | Mint." Accessed: Sep. 05, 2023. [Online]. Available: https://www.livemint.com/science/health/over-100-cases-of-tomato-flu-detected-in-children-under-9-years-of-age-health-ministry-issues-advisory-11661265237625.html
16. "India logs 82 cases of 'Tomato Fever' in children below 5 years, Lancet issues alert | Mint." Accessed: Sep. 23, 2023. [Online]. Available: https://www.livemint.com/science/health/india-logs-82-cases-of-tomato-fever-in-children-below-5-years-lancet-issues-alert-11660992908375.html
17. "Tomato fever grips Kerala, Tamil Nadu steps up vigil on border | India News | Zee News." Accessed: Sep. 23, 2023. [Online]. Available: https://zeenews.india.com/india/tomato-fever-grips-kerala-tamil-nadu-steps-up-vigil-on-border-2463348.html
18. "Tomato fever' is misleading name for Hand Foot and Mouth Disease, says expert | Mint." Accessed: Sep. 23, 2023. [Online]. Available: https://www.livemint.com/news/india/tomato-fever-is-misleading-name-for-hand-foot-and-mouth-disease-says-expert-11661076331926.html
19. A. Ismail, A. Saahath, Y. Ismail, M. F. Ismail, Z. Zubair, and K. Subbaram, "'Tomato flu' a new epidemic in India: Virology, epidemiology, and clinical features," *New Microbes and New Infections*, vol. 51. Elsevier Ltd, Jan. 01, 2023. doi: 10.1016/j.nmni.2022.101070.
20. G. M. Sickles, M. Mutterer, P. Feorino, and H. Plager, "Recently Classified Types of Coxsackie Virus, Group A. Behavior in Tissue Culture," *Proceedings of the Society for Experimental Biology and Medicine*, vol. 90, no. 2, pp. 529–531, 1955, doi: 10.3181/00379727-90-22088.
21. Q. Mao *et al.*, "Coxsackievirus A16 Epidemiology, diagnosis, and vaccine," *Hum Vaccin Immunother*, vol. 10, no. 2, pp. 360–367, 2014, doi: 10.4161/hv.27087.
22. M. Gong *et al.*, "Cryo-Electron Microscopy Study of Insect Cell-Expressed Enterovirus 71 and Coxsackievirus A16 Virus-Like Particles Provides a Structural Basis for Vaccine Development," *J Virol*, vol. 88, no. 11, pp. 6444–6452, Jun. 2014, doi: 10.1128/jvi.00200-14.
23. Z. Du *et al.*, "Association of neighborhood greenness with severity of hand, foot, and mouth disease," *BMC Public Health*, vol. 22, no. 1, Dec. 2022, doi: 10.1186/s12889-021-12444-7.
24. S. Y. Fong *et al.*, "A five-year retrospective study on the epidemiology of hand, foot and mouth disease in Sabah, Malaysia," *Sci Rep*, vol. 11, no. 1, Dec. 2021, doi: 10.1038/s41598-021-96083-3.
25. Q. Gao *et al.*, "Forecast and early warning of hand, foot, and mouth disease based on meteorological factors: Evidence from a multicity study of 11 meteorological geographical divisions in mainland China," *Environ Res*, vol. 192, Jan. 2021, doi: 10.1016/j.envres.2020.110301.

26. P. Sean and B. L. Semler, "Coxsackievirus B RNA Replication: Lessons from Poliovirus The encoded nonstructural proteins are responsible for RNA replication through."
27. A. Ismail, A. Saahath, Y. Ismail, M. F. Ismail, Z. Zubair, and K. Subbaram, "'Tomato flu' a new epidemic in India: Virology, epidemiology, and clinical features," *New Microbes and New Infections*, vol. 51. Elsevier Ltd, Jan. 01, 2023. doi: 10.1016/j.nmni.2022.101070.
28. R. E. Sanjay *et al.*, "Molecular epidemiology of enteroviruses associated with hand, foot, and mouth disease in South India from 2015 to 2017," *Arch Virol*, vol. 167, no. 11, pp. 2229–2238, Nov. 2022, doi: 10.1007/s00705-022-05561-0.
29. Q. Niu *et al.*, "Explanation of hand, foot, and mouth disease cases in Japan using Google Trends before and during the COVID-19: infodemiology study," *BMC Infect Dis*, vol. 22, no. 1, Dec. 2022, doi: 10.1186/s12879-022-07790-9.
30. A. Mirand *et al.*, "A large-scale outbreak of hand, foot and mouth disease, France, as at 28 September 2021," *Euro Surveill*, vol. 26, no. 43, Oct. 2021, doi: 10.2807/1560-7917.ES.2021.26.43.2100978.
31. X. D. Meng *et al.*, "Epidemical and etiological study on hand, foot and mouth disease following EV-A71 vaccination in Xiangyang, China," *Sci Rep*, vol. 10, no. 1, Dec. 2020, doi: 10.1038/s41598-020-77768-7.
32. P. Mcminn, I. Stratov, L. Nagarajan, and S. Davis, "Neurological Manifestations of Enterovirus 71 Infection in Children during an Outbreak of Hand, Foot, and Mouth Disease in Western Australia," 2001. [Online]. Available: https://academic.oup.com/cid/article/32/2/236/319948
33. N. Martínez-López *et al.*, "Surveillance for enteroviruses associated with hand, foot, and mouth disease, and other mucocutaneous symptoms in Spain, 2006–2020," *Viruses*, vol. 13, no. 5, May 2021, doi: 10.3390/v13050781.
34. C. Luo, J. Qian, Y. Liu, Q. Lv, Y. Ma, and F. Yin, "Long-term air pollution levels modify the relationships between short-term exposure to meteorological factors, air pollution and the incidence of hand, foot and mouth disease in children: a DLNM-based multicity time series study in Sichuan Province, China," *BMC Public Health*, vol. 22, no. 1, Dec. 2022, doi: 10.1186/s12889-022-13890-7.
35. R. R. Kashyap and R. S. Kashyap, "Hand, foot and mouth disease - a short case report," *J Clin Exp Dent*, vol. 7, no. 2, pp. e336–e338, 2015, doi: 10.4317/jced.52031.
36. J. Guo *et al.*, "Epidemiology of hand, foot, and mouth disease and the genetic characteristics of Coxsackievirus A16 in Taiyuan, Shanxi, China from 2010 to 2021," *Front Cell Infect Microbiol*, vol. 12, Nov. 2022, doi: 10.3389/fcimb.2022.1040414.
37. S. Esposito and N. Principi, "Hand, foot and mouth disease: Current knowledge on clinical manifestations, epidemiology, aetiology and prevention," *European Journal of Clinical Microbiology and Infectious Diseases*, vol. 37, no. 3. Springer Verlag, pp. 391–398, Mar. 01, 2018. doi: 10.1007/s10096-018-3206-x.
38. S. A. Pattassery *et al.*, "Molecular epidemiology of hand, foot, and mouth disease in Karnataka, India in 2022," *Indian J Med Microbiol*, vol. 46, p. 100429, Nov. 2023, doi: 10.1016/J.IJMMB.2023.100429.
39. P. Zhu *et al.*, "Current status of hand-foot-and-mouth disease," *Journal of Biomedical Science*, vol. 30, no. 1. BioMed Central Ltd, Dec. 01, 2023. doi: 10.1186/s12929-023-00908-4.
40. Y. Zhang and W. B. Xu, "Molecular epidemiology of enteroviruses associated with hand, foot, and mouth disease in the mainland of China," *Biomedical and Environmental Sciences*, vol. 26, no. 11, pp. 875–876, Nov. 2013, doi: 10.3967/bes2013.015.
41. J. Hong *et al.*, "Changing epidemiology of hand, foot, and mouth disease in China, 2013À2019: a population-based study," *Lancet Reg Health West Pac*, vol. 20, p. 100370, 2022, doi: 10.1016/j.
42. M. Chen *et al.*, "Severe hand, foot and mouth disease associated with Coxsackievirus A10 infections in Xiamen, China in 2015," *Journal of Clinical Virology*, vol. 93, pp. 20–24, Aug. 2017, doi: 10.1016/j.jcv.2017.05.011.
43. "Hand-Foot-Mouth Disease caused by Coxsackievirus A16 increases in Assam." Accessed: Oct. 31, 2023. [Online]. Available: https://assamtribune.com/assam/hand-foot-mouth-disease-caused-by-coxsackievirus-a16-increases-in-assam-1445520?infinitescroll=1

44. N. Knöpfel, L. Noguera-Morel, I. Latour, and A. Torrelo, "Viral exanthems in children: A great imitator," *Clin Dermatol*, vol. 37, no. 3, pp. 213–226, May 2019, doi: 10.1016/j.clindermatol.2019.01.009.
45. "Home :: Integrated Disease Surveillance Programme(IDSP)." Accessed: Nov. 05, 2023. [Online]. Available: https://idsp.mohfw.gov.in/
46. A. S *et al.*, "Tomato Flu- A Review on Existing Scenario," *Int. J Pharm Sci Rev Res*, pp. 196–199, Aug. 2022, doi: 10.47583/ijpsrr.2022.v75i02.032.
47. P. Paul, S. Paul, N. Era, K. L. Barik, and U. K. Paul, "Tomato Flu- A New Virus Trending in Children," *JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH*, 2023, doi: 10.7860/jcdr/2023/61125.18069.
48. A. A. L. Song, L. L. A. In, S. H. E. Lim, and R. A. Rahim, "A review on *Lactococcus lactis*: From food to factory," *Microbial Cell Factories*, vol. 16, no. 1. BioMed Central Ltd., Apr. 04, 2017. doi: 10.1186/s12934-017-0669-x.
49. Z. Jiang, X. Tian, X. Lu, and B. Zhong, "An oral vaccine against CVA16 (Coxsackievirus A16) was developed by constructing a recombinant *Lactococcus lactis*," *Tropical Journal of Pharmaceutical Research*, vol. 19, no. 5, pp. 927–932, May 2020, doi: 10.4314/tjpr.v19i5.3.
50. Y. S. Sun *et al.*, "Development and evaluation of an inactivated coxsackievirus A16 vaccine in gerbils," *Emerg Microbes Infect*, vol. 11, no. 1, pp. 1994–2006, 2022, doi: 10.1080/22221751.2022.2093132.

