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Research Article

Characteristics of Non-Polio Enterovirus Associated Acute Flaccid Paralysis Among Children in Sri Lanka: A Retrospective Study

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Abstract

Background:

In the era of polio on the verge of eradication, non-polio enteroviruses (NPEVs) have frequently been identified as etiological agents of acute flaccid paralysis (AFP), necessitating greater attention to their epidemiology and transmission dynamics. AFP surveillance has shown to be a valuable source for tracking NPEV-associated paralysis and understanding emerging patterns of infection. However, in Sri Lanka, the epidemiology of NPEVs remains largely unexplored. This study aims to investigate the characteristics of NPEV in children with AFP, and to understand their spatial and temporal distribution across the country. Acute Flaccid Paralysis (AFP) is a critical clinical syndrome characterized by sudden limb weakness and floppiness, which can lead to severe complications, including respiratory failure. Traditionally, poliovirus has been a major cause of AFP, but the global eradication efforts have highlighted other pathogens, particularly non-polio enteroviruses (NPEVs). Understanding the demographic, clinical, and geographic distribution of NPEV-associated AFP cases is crucial for effective surveillance and response strategies, especially in post-polio eradication context.

Methodology:

The study retrospectively analyzed 376 children under the age of 15 years with acute flaccid paralysis (AFP), whose samples were sent for poliovirus and NPEV isolation using L20B and RD cell lines at the Polio Regional Reference Laboratory of the Medical Research Institute, between January 2019 and December 2023.

Results:

Of the 376 AFP cases analyzed, 27 (7.2%) were positive for NPEV, while 6 (1.6%) were positive for vaccine-derived poliovirus. The annual NPEV isolation rate varied between 4.0% and 13.4%, with a predominance in male patients (62.8%) and the highest incidence observed in children aged 1–5 years (70.4%). Notably, 92.6% of NPEV-positive cases had documented vaccination history. NPEV cases were detected in 7 out of 9 provinces, with the Western Province reporting the highest number of cases (40.7%) across all study years. Seventy four percent of NPEV cases were concentrated in the Southern, Western, and Central provinces. Seasonal trends in virus transmission were observed, with peak isolation occurring between May and July in the Southwest and Central regions, followed by a secondary peak from October to November in the North-Eastern regions

Conclusions:

NPEV is an emerging cause of AFP among younger children in Sri Lanka. The finding highlights a widespread circulation of

NPEVs in Sri Lanka, with distinct seasonal and regional patterns, underscoring the need for enhanced AFP surveillance to monitor NPEV transmission and mitigate potential outbreaks.

Key words: acute flaccid paralysis; non-polio enterovirus; sri lanka

Introduction

Acute flaccid paralysis (AFP) is a clinical syndrome, manifests as the sudden onset of limb weakness and floppiness, accompanied by diminished muscle tone, primarily affecting children under 15 years of age. The condition typically follows a progressive course, but has potential to transition into a chronic state. In severe cases, AFP can lead to life-threatening complications, particularly if paralysis extends to the diaphragm, resulting in respiratory failure. The AFP has a diverse range of etiologies, including both infectious (viral and bacterial) and non-infectious causes. Among these Guillain-Barre syndromes (GBS) is being reported as the most common cause worldwide (The National Institute for Communicable Diseases, 2020). Historically, polio virus, a member of Enterovirus C, was the most debilitating cause of AFP, particularly in young children. However, in the light of activities of the Global Polio Eradication Initiative (GPEI), launched by the WHO in 1988, the global incidence of poliovirus has reduced to 99.9% through extensive immunization programmes and active surveillance systems. As a result, wild poliovirus (WPV) has been eradicated in Sri Lanka, with the last reported case in 1993, and vaccine-associated poliomyelitis is also expected to be eliminated in the near future. Despite these achievements, AFP remains a major contributor to neuromotor impairment in children across Sri Lanka. In recent years, non-polio enteroviruses (NPEVs; family Picornaviridae) have emerged as a key driver of polio-like epidemics. Most of these NPEV infections are associated with severe neurological complications, particularly in young children, often occurring in geographically clustered outbreaks with a distinct biennial seasonal pattern in temperate regions. Among these, Enterovirus D68 (EV-D68) is suspected to be the predominant causative agent of seasonal AFP outbreaks, though other enteroviruses such as EV-A71 and E30, along with certain Coxsackievirus strains, have also been implicated. In the post-polio era, EV- associated AFP is expected to become increasingly prominent. In this scenario, strengthening AFP surveillance at both regional and national levels is imperative to facilitate the early detection of virus importation or local emergence and ensure a rapid public health response. Several developed countries have established national surveillance programs to monitor circulating NPEVs through laboratory-based epidemiological data. However, Sri Lanka currently lacks a dedicated NPEV surveillance program, relying solely on its national AFP surveillance system, which was originally designed to monitor poliovirus as part of the Global Polio Eradication Initiative (GPEI) in 1991. It identifies AFP cases nationwide through systematic case detection of patients presenting with polio-like symptoms, followed by biological sample analysis to differentiate between wild-type (WPV) and vaccine-derived poliovirus (VDPV). Concurrently, this AFP framework also enables the monitoring of NPEV circulation within the community, offering a crucial, albeit indirect, insight into the epidemiology of non-polio AFP in Sri Lanka. Given the rising global burden of NPEV-associated AFP and the lack of epidemiological data in Sri Lanka, we conducted a study to examine the prevalence, sociodemographic patterns, and geographical distribution of NPEV-associated AFP cases reported to AFP surveillance system from 2019 to 2023. The findings will provide critical insights into the evolving epidemiology of AFP, assess the effectiveness of the current surveillance system, and identify gaps in NPEV detection and monitoring. Strengthening surveillance is essential for early detection, outbreak preparedness, and sustaining Sri Lanka's progress toward an NPEV-AFP- free status.

Methods

Ethical statement

This study retrospectively analyzed laboratory data derived from stool specimen cultures of AFP cases sent for routine AFP and VDPV surveillance at the Polio Regional Reference Laboratory, Medical Research Institute of Sri Lanka, Colombo, from 2019 to 2023. As a WHO accredited laboratory within global polio network, the Polio Regional Reference Laboratory stands as the premier center for evaluating AFP case specimens nationwide. Since the study extracted data from the national AFP surveillance system, ethical approval was waived off by the Ethics Review Committee of the Medical Research Institute. All the measures were taken to ensure data privacy and confidentiality, fully adhering to national regulatory frameworks. Given the retrospective nature of the study and the absence of personally identifiable information, obtaining patient consent was deemed unnecessary.

Data collection

The study included surveillance data obtained from 376 children under 15 years of age who clinically manifested AFP symptoms, as defined by AFP surveillance programme between January 2019 to December 2023. Cases with missing data, insufficient stool samples for laboratory analysis, or patients who had not been followed up were excluded from the study. All the clinically diagnosed AFP cases all over the country has to be reported health authorities and clinical samples (two stool specimens per case) collected within 14 d onset of paralysis and 24 apart refer to the Polio Regional Reference Laboratory, MRI. All the clinical samples for AFP were processed for virus isolation and reported according to the WHO standard procedures. Upon receipt to the laboratory, stool samples were inoculated into LB20 (human poliovirus receptor-CD155 expressing recombinant murine cells) cell lines and RD (human rhabdosarcoma) cell lines, and then examined for cytopathic effects (CPE) distinguish NPEV from poliovirus. Viral cultures exhibiting CPE only in RD cell lines were considered as NPEV, whereas those showing CPE in both L20B and RD cell lines were classified as positive for poliovirus.

Data analysis

Data was analyzed using Statistical Package for Social Science (SPSS) version 21 (IBM SPSS Inc. Chicago, USA). Descriptive statistics were used to characterize NPEV cases. The following information was extracted from the laboratory data system: results of virus isolation from L20B and RD cell lines, socio-demographic data including age and gender. Identification of NPEV cases were based on the viral inoculation in L20B and RD cell lines for virus detection and differentiation of polio virus and NPEV. In NPEV positive cases, cytopathic effects (CPE) were observed only in RD cell lines, whereas in polio positives, CPEs were detected in both L20B and RD cell lines. The study followed guidelines in Polio Laboratory Manual, 4th ed. (2004) WHO to collect above data. Data were entered on Microsoft excel sheet and analyzed using Statistical Package for Social Science (SPSS) version (IBM SPSS statistics version 21). Descriptive statistics were used to explain characteristics of NPEV cases.

Results

A total of 376 AFP cases were reported between 2019 and 2023, with an annual number of cases exceeding 70, except for a notable decline in 2020 (50 cases). Over the five-year period, NPEV was detected in 27 cases (27/376, 7.2%), while VDPV was identified in six cases (06/376, 1.6%) (Table 1.1). 1 NPEV isolation rates and clinical characteristics of NPEV associated AFP cases Between 2019 and 2023, the annual NPEV positivity rate exhibited notable fluctuations, particularly during the period of the SARS-CoV-2 pandemic. In 2019, the NPEV positivity rate reached a peak of 13.4%. However, despite a substantial number of reported AFP cases in the subsequent years, the NPEV isolation rate declined to below 4% during the 2020–2022 period, coinciding with the COVID-19 pandemic. A resurgence in NPEV detection was observed in 2023, with the positivity rate rising to 11.6%, suggesting a possible rebound in enterovirus circulation. Among AFP cases positive for NPEV, the incidence was significantly higher

in male patients (17, 62.9%) compared to females (10, 37.0%), with a male to female ratio of 1.7:1. The highest infection rate was observed in children aged 1–5 years (19, 70.4%), with a gradual decline in incidence as age increased. The lowest number of NPEV infections was found in infants under 12 months (1, 3.7%). Fever at the onset of paralysis was reported in 51.9% (14/27) of NPEV associated AFP cases while no reliable information was available for patients having fever or not (11, 40.7%) due to missing data. Further, 92.6% of NPEV cases had received polio vaccine, and 7.4% were unaware of their polio immunization status (Table 1.3).

	Frequency (n=376)	Percentage (%)
Polio(vaccinederived)	6	1.6
NPEV	27	7.2
Negativefor polio and NPEV	343	91.2

Table 11: Frequency of polio and non-polio enterovirus cases reported over the period of 2019-2023 based on the polio cell culture analysis of stool samples from acute flaccid paralysis patients.

The annual positivity rate of NPEV associated AFP cases from 2019 to 2023 is given under table 1.2 The highest NPEV-AFP positivity rate of 13.41% was reported in 2019 and considerably similar second highest was reported in 2023 as 11.58%. In 2020 and 2022 NPEV-AFP positivity rates were reported as 4.0% and 3.8% respectively. In 2021 the positivity rate was reported as 0% percentage.

	2019	2020	2021	2022	2023
Number of NPEV-AFP cases reported	11	2	0	3	11
NPEV- AFP positivity rate	13.41%	4.0%	0.0%	3.80%	11.58%

Table 1.2: Annual positivity rate of NPEV associated AFP cases from 2019-2023.

Characteristic	Frequency (n=27)	Percentage from total NPEV cases n=27 (%)
Gender		
Female	10	37.04
Male	17	62.96
Age		
<1 year	1	3.70
1-5 year	19	70.37
6-10 year	5	18.51
11-15 year	2	7.40
Fever at onset of AFP	14	51.85
Yes	2	7.41
No Unknown	11	40.74
Vaccinated status	25	92.59
Yes No	0	0
Unknown	2	7.41

Table 1.3: Frequency Demographic and clinical characteristics of NPEV associated AFP cases.

2.Seasonal distribution of NPEV associated AFP cases

During the evaluation period from 2019 to 2023, AFP cases were consistently reported throughout the year, with a mean monthly incidence of [31.3] cases and no significant seasonal variation in case distribution, indicating a stable occurrence pattern over time. figure 2.1. In contrast, the NPEV infection showed a seasonality in monthly distribution, with more prominent three peaks: May to June (40.74%), in August, (11.11%) and from October to November (33.33%). The number of NPEV cases were dropped notably between December and April, while no single case was reported in March, September and December for all cases from 2019 to 2023. However, when considering the AFP-NPEV cases, a significantly greater number of cases were observed in the month of May accounting for 22.2% of the NPEV cases. Other months with a high number of cases included June, October, November and August accounting for 18.5%, 18.5%, 14.8% and 11.1% of NPEV cases, respectively. The seasonal distribution of NPEV positive cases in the study population 2019 to 2023 is illustrated in figure 2.2.

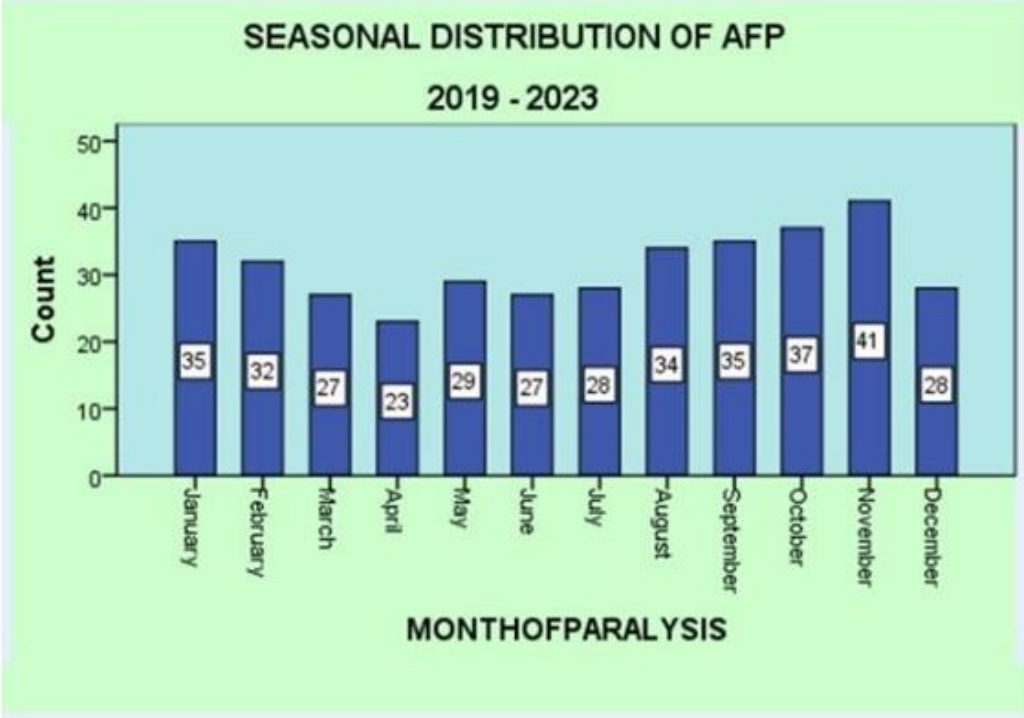

Figure 2.1: Seasonal distribution of AFP cases from 2019 – 2023.

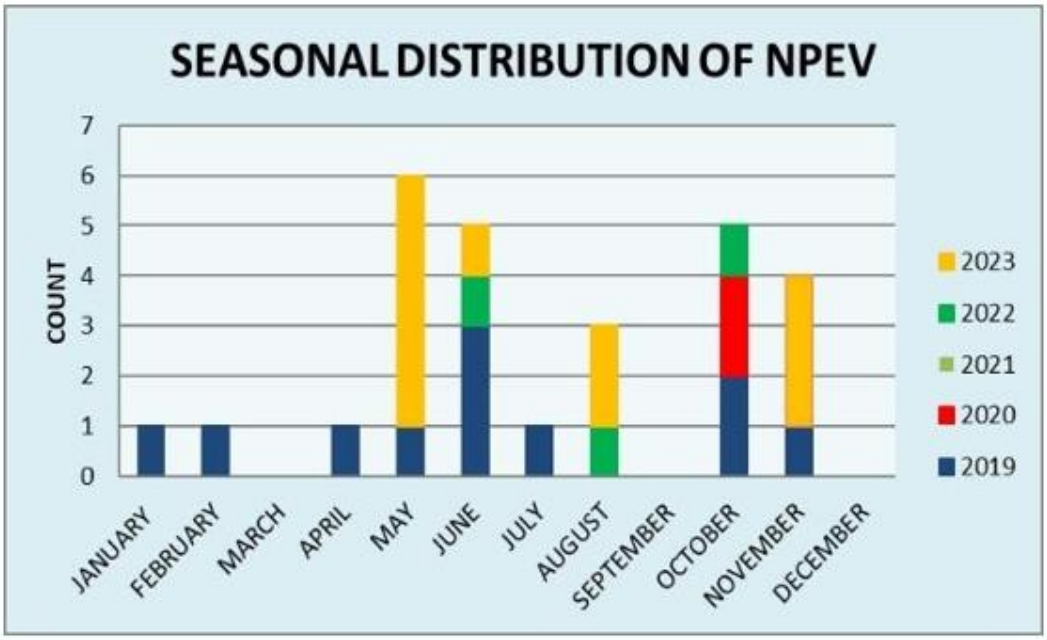

Figure 2.2: Monthly distribution of NPEV cases in the AFP study population, 2019 – 2023.

3.Geographical distribution of NPEV associated AFP cases in Sri Lanka from 2019 - 2023

Sri Lanka is administratively divided into nine provinces, which are further subdivided into 26 districts. The figure 3.1 illustrates the geographical distribution of NPEV-AFP cases at the provincial level, highlighting notable regional variations in case burden. The highest case load was reported from Western Province (11, 40.74%), with Kalutara District accounting for the highest number of cases (06, 22.22%), followed by Colombo (04, 14.81%) and Gampaha (01, 3.7%). The Southern Province had the second highest case burden (05,18.51%) distributed across Galle (03) and Matara (02) Districts. NPEV case detection was comparatively low in Uva (01) and Northern (01) Provinces, each with only one case (3.70%). No NPEV infection was detected in and North Central Provinces for the 5 years of study period. The district-level distribution of NPEV-positive cases is detailed in Figure 3.2. Sri Lanka, an equatorial island, experiences distinct climatic variations across its regions due to its diverse topography, leading to significant differences in rainfall, temperature, and wind patterns that may influence the circulation of NPEVs. Therefore, we analyzed the monthly distribution of NPEV-AFP cases across different provinces, with the findings presented in Figure 3.3. The evaluation of NPEV peak during the May-June, cases were predominantly reported from the Western (4), Southern (4), and Central Provinces (2). In the July-August peak, cases were detected in the Western (2), Southern (1), and Northwestern (1) Provinces. During the October-November peak, NPEV isolation was notable in Western (4), Uva (1),

Northwestern (1), and Northern (1) Provinces. Overall, a clear spatial trend was observed in the distribution of NPEV cases during the seasonal peaks. The May–August period accounted for the highest annual NPEV case burden, with viral circulation predominantly concentrated in the South-West regions of Sri Lanka. In contrast, during the October–November peak, there was a notable shift in NPEV case distribution toward the North-East coastal areas. During all seasonal peaks, the Western Province consistently reported the highest number of cases, highlighting its role as a key focal point for NPEV circulation in Sri Lanka.

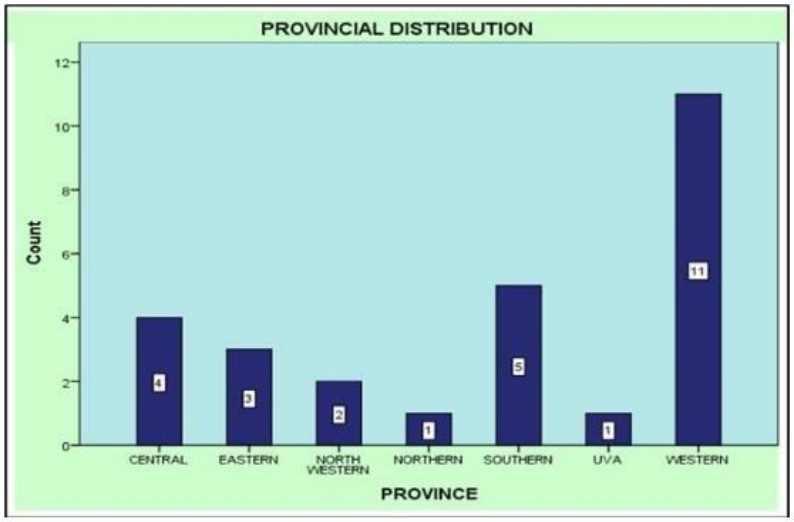

Figure 3.1: Geographical distribution of NPEV cases at provincial level 2019 – 2023.

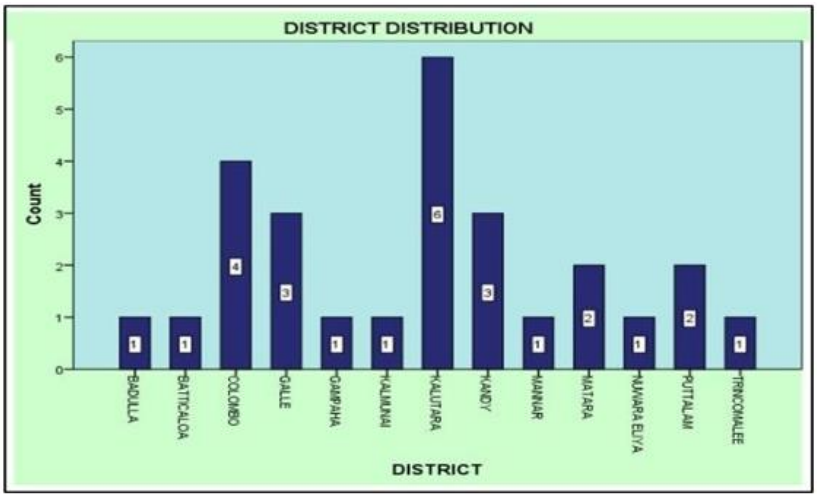

Figure 3.2: Geographical distribution of NPEV cases at district level 2019 – 2023.

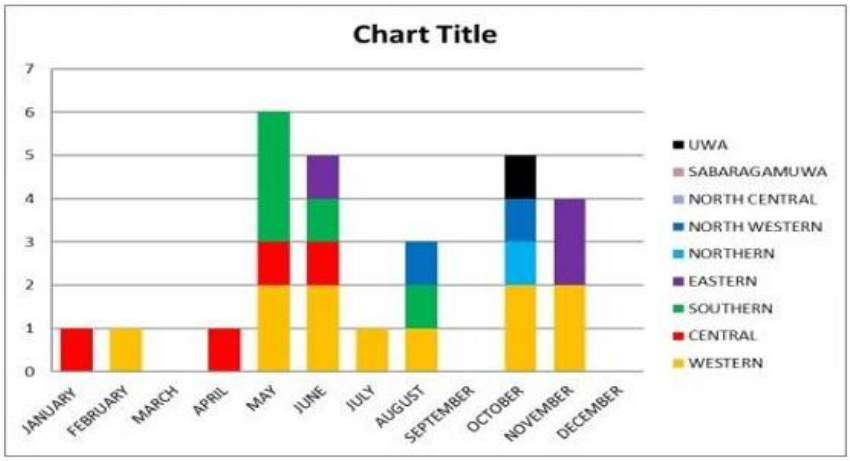

Figure 3.3: Frequency of NPEV-associated AFP cases reported at the provincial level each month (2019-2023).

Discussion

To date, Sri Lanka lacks a dedicated hospital or community-based NPEV surveillance system, making AFP surveillance the primary mechanism for detecting and monitoring NPEV circulation. To the best of our knowledge, no comprehensive epidemiological data on NPEV infections have been documented over the past five years, despite the growing annual incidence of AFP cases in Sri Lanka. This study investigated the NPEV isolation rates in AFP cases and analyzed their distribution across Sri Lanka, providing critical insights into the epidemiological characteristics of these infections at the local level. During the five-year study period, the NPEV isolation rate among AFP cases in children <15 years fluctuated between 4% and 13%, with an unexpected drop in 2020, 2021, and 2022, coinciding with the implementation of stringent COVID-19 restriction measures. However, the resurgence of NPEV cases in 2023, reaching 12%, suggests that under normal circumstances, the annual NPEV derived AFP cases in Sri Lanka expected to be ranged between 10% and 13%, slightly exceeding the 10% NPEV isolation rate recommended by WHO to the reference laboratories. However, the frequency of NPEV isolation in our study is much lower than other Asian countries, such as India, Pakistan, Philippines, and South Korea, where NPEV detection rates have been reported between 19% and 34%. Additionally, 1.6% of AFP cases in this study resulted positive to VDPV in line with the national epidemiological reports. NPEV isolation rates below 10% may indicate low circulation of the virus within the country. Additionally, several factors could also contribute to the reduced detection rate, including potential limitations in the sensitivity of isolation techniques or suboptimal conditions during sample collection, storage, and transportation, which may affect viral viability, particularly in topical countries. The epidemiological characteristics of NPEV-associated AFP cases in this study align with findings from the current scientific literature, with the majority of affected patients being male (62.96%), younger than 5 years (70.37%). These results are close to the findings reported in studies conducted in South Korea, Pakistan and Philippine, which have shown a heightened vulnerability to NPEV-associated AFP among children under five years of age (Yoon et al., 2021; Jiao et al., 2020; Saeed et al., 2007). However, the isolation rate among children <5 years appear to vary across different regions, with lower values reported in South -Western India, Indonesia (Tushabe et al., 2021) Our study observed a high vaccination coverage among AFP cases, with 92.59% of the total population having received PV vaccine. The remaining 7.41% had undocumented vaccination status, suggesting that the actual coverage rate could be even higher and reach the target of 95% as the recommended polio vaccination coverage to prevent the reintroduction of the virus. This finding supports the effectiveness of the National Immunization Programme in Sri Lanka. The detection of NPEV across seven out of nine provinces and 13 out of 26 districts suggests the widespread circulation of EV viruses throughout Sri Lanka. However, significant spatial variations in NPEV distribution were observed, with the highest burden reported in Kalutara and Colombo Districts within the Western Province. Notably, NPEV isolation was more prevalent in the Western-South coastal belt and the Central Province, particularly during the months of May to June, coinciding with the start of the Southwest Monsoon (May–September). Conversely, NPEV detection was lower in the Northern and Eastern coastal regions, which fell within Sri Lanka's dry zone. However, a surge in NPEV activity was noted in these regions during the months of October to November, aligning with the Second Inter-Monsoon period. This seasonal variation implies that monsoonal rainfall, and other climatic factors including humidity, wind, and associated environmental changes may influence NPEV transmission dynamics. Interestingly, a considerable number of NPEV cases were also detected in districts such as Kandy, Badulla, and Nuwara Eliya regions characterized by cooler climates and higher altitudes. This observation suggests that, while temperature and humidity play a role in NPEV dynamics, viral persistence and transmission may also be sustained in non-coastal, high-altitude regions, potentially through alternative mechanisms such as human mobility, environmental reservoirs, or localized outbreaks. limitations of the study- The present study observed AFP cases throughout the year without any significant difference, but NPEV-AFP cases were higher during warm months with high humidity. Similar results were reported studies from Northern India, South Western India and South Korea (Yoon et al., 2021). The present study findings revealed NPEV-AFP cases detected significantly in three seasonal peaks; a greater number reported during months, start of the Southwest Monsoon (40.74%, May-June) rather than monsoon months (11.11%, August) and second inter monsoon months (33.33%, October-November). Northern-east monsoon months (December- February) reported considerably smaller number of cases. Sri Lanka experiences two main monsoon seasons- the Southwest monsoon (May –September) and the Northeast monsoon as well as two inter monsoonal periods. First inter monsoon (March-April) and second inter monsoon (October-November) periods occur between main monsoon seasons. This study suggests NPEV-AFP generally spread more during Warm and humid conditions, it might be due to increase social contacts during summer months and enhance the virus stability and survival on surfaces, aiding transmission. (User, 2019) The geographic distribution of NPEV-AFP reported across 07 provinces including 13 districts in Sri Lanka. A significant percentage (40.74%) of cases were concentrated in Western province with the highest percentage (22.22%) reported from Kalutara district. Factors such as population density, and hygiene practices might contribute to the geographical differences observed in the incidence of NPEV-associated AFP. This study analysis shows that NPEV cases are significantly reported from South West area during May-June peak while the October-November peak has higher cases from North-East coastal areas of Sri Lanka.

Conclusion

This study contributes to establish 7.2% of AFP-NPEV positivity among children aged under 15 years in Sri Lanka 2019 - 2023. The current study suggests that in post-polio era, AFP associated NPEV positive cases will continue to emerge, and identification procedures should be prioritized. The findings of this study show characteristics of NPEV gives better understanding of NPEV associated AFP and could be used for further studies on NPEV circulating in Sri Lanka and establish more effective strategies to prevent NPEV. In the post-polio era, NPEVs is identify as a significant cause of AFP in Sri Lanka, particularly among young children. While NPEV circulation is widespread across the country, it is notably concentrated in the Western, Southern, and Central regions, with peak isolation occurring during the May–June and October–November periods, likely influenced by monsoonal climate variations. Strengthening AFP surveillance with enhanced indicators for NPEV detection, coupled with a robust data management system, is crucial for a comprehensive understanding of NPEV-associated AFP, tracking circulating lineages, and implementing timely interventions to mitigate potential future outbreaks. This study indicates the need for improved record-keeping. Also, this highlights critical demographic trends among AFP cases, pointing to the necessity for ongoing monitoring and intervention strategies, particularly for young children. Overall, this study highlights critical insights into the epidemiology of AFP and NPEV cases in Sri Lanka, emphasizing the need for continued surveillance, vaccination efforts, and improved documentation practices. Future research

should focus on clarifying the clinical and environmental factors contributing to the observed seasonal peaks and geographical distribution, ultimately aiding in the development of targeted public health strategies.

Declaration of of Competing Interest

Authors would like to declare that there are no potential competing interests.

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