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Open Access Research Article

Non-Polio Enteroviruses in Acute Flaccid Paralysis: Analysis of Surveillance in Iraq from 2018 to 2024

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Abstract

Background: Acute flaccid paralysis surveillance, forms the basis for poliovirus eradication efforts and provides useful data on non-polio enteroviruses, which are significant causes of acute flaccid paralysis in polio-free countries. Non polio enteroviruses were the most common isolates from acute flaccid paralysis cases at this time, indicating sensitivity of surveillance and continued circulation of enteroviruses.
Objectives: This study aimed to determine the prevalence, epidemiologic trends and clinical features of non- polio enteroviruses among children with acute flaccid paralysis in Iraq, from 2018 to 2024, for improving surveillance sensitivity and complementary evidence-based public health measures.
Methods: The data was gathered from the acute flaccid paralysis surveillance system in Iraq. Stool specimens were analyzed at the National Polio Laboratory according to the World Health Organization guidelines. Suspensions of stool were prepared and inoculated into the Rhabdomyosarcoma cell line and genetically modified mouse L cells expressing the human poliovirus receptor. Isolates were subjected for real-time polymerase chain reaction, whereas cultures were checked for cytopathic effect. Isolates negative for poliovirus but positive in Rhabdomyosarcoma cells were classified as non-polio enteroviruses. Demographics and clinical findings were analyzed using appropriate statistical tests.
Results: Non-polio enteroviruses were reported in 10.5% of the cases; the prevalence being highest in children 1 to 2 years. They appeared to be two peaks; one in January and another in May, and cases were particularly low in September. Non-polio enteroviruses were associated with residual weakness and lower mortality.
Conclusions: Non-polio enteroviruses detection declined during 2020–2023 with recovery in 2024. Positivity was significantly higher in younger children, and demonstrated clear seasonal peaks. Non-polio enteroviruses infection was associated with residual weakness but not with gender, fever, or mortality.

Keywords: Acute flaccid paralysis, Demographic characteristics, Iraq, mortality, Non-Polio Enteroviruses, residual weakness, surveillance.

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Introduction

Acute flaccid paralysis (AFP) remains an important global public health issue because of its association with poliovirus, a pathogen targeted for worldwide eradication. AFP, characterized by sudden onset of flaccid paralysis, is most frequently linked to poliomyelitis. The Global Polio Eradication Initiative (GPEI) has significantly decreased the percentage of AFP attributable to the poliovirus, shifting the focus towards other non-polio enterovirus (NPEVs), as a major cause of AFP in polio-free countries (1). Belonging to the family Picornaviridae, NPEVs encompass >100 serotypes of Coxsackieviruses, Echoviruses and Enteroviruses (2,3). Recent studies have demonstrated significant neurological implications, especially in children, underscoring the need for continued monitoring of AFP cases during and after follow up (4). The World Health Organization (WHO) emphasizes on the importance of AFP surveillance for NPEVs, to assess their prevalence and identify the immunity gaps (5). However, in countries that are already polio-free, such as Iraq, sporadic AFP cases serve as a public health issue. Effective surveillance systems are imperative, to differentiate poliovirus from other etiologies and NPEVs. NPEVs have exhibited seasonal fluctuation, which is higher during warmer periods, owing to high viral transmission. After importation of wild poliovirus from Syria in 2014, regional polio-free certification occurred in September 2015 and WHO certified Iraq as polio-free country in 2017(6,7,8). In the meantime, AFP surveillance was reinforced onward by enhancing both case detection and timely reporting as well as stool adequacy rates. Iraq, however, steadily reached important performance indicators indicated by the WHO as routinely measuring how well surveillance for non-polio AFP is conducted, including a non-polio AFP rate ≥2 per 100000 children under 15 years and stool adequacy≥80%, indicating system strengthening and gradual recovery (9). Iraq has remained polio-free as of 2018, and between 2018 and the end of 2024, they reported no wild poliovirus cases. Non-polio enteroviruses are the most prevalent viral isolates in AFP cases and considered an important measure of fecal–oral transmission coupled with surveillance quality. In this period, surveillance indicators were stable while laboratory capacity and data management systems improved, providing a solid basis for assessing age-specific patterns and epidemiology of NPEVs in Iraq (10).

Global health systems and programs, including AFP surveillance and immunization programs, have also been severely impacted by the COVID-19 pandemic. Reduced surveillance activities worldwide, during this period, might have led to an underestimation of the actual burden of the AFP and NPEV cases (11). The study period was selected to reflect a stage in the post-polio-free certification era when surveillance indicators were stable and allowed reliable estimates of non-polio enterovirus (NPEV) epidemiology. The epidemiological analysis of demographic and seasonal patterns of NPEVs help in estimating the contribution of NPEVs towards AFP, as well as provide appropriate data to formulate public health strategies for better control (12, 13). This study aimed to determine the prevalence, epidemiologic trends and clinical features of Non-polio enteroviruses among the acute flaccid paralysis cases in Iraqi children less than 15 years of age, between 2018 and 2024, for improving surveillance sensitivity and complementary evidence-based public health measures.

Patients and Method

This retrospective study used data from Iraq's national acute flaccid paralysis (AFP) surveillance system, including all AFP cases in children <15 years with onset of paralysis between January 2018 and December 2024. AFP is a notifiable disease and reported weekly from an integrated national surveillance network. Details of demographics, clinical features, laboratory findings and outcome were documented in the national database system. Within 48 hours of the investigation, AFP cases were handled. Within 14 days after onset, two stool specimens (8–10 g) were collected with an interval of 24–48 hours. Contacts and suboptimal cases additional samples were obtained. Specimens were delivered through reverse cold chain (2–8°C) to the National Polio Laboratory.

Laboratory procedures adhered to World Health Organization protocols. Suspensions of stool were prepared and inoculated into the Rhabdomyosarcoma cell line (human rhabdomyosarcoma–derived cell line used in poliovirus surveillance because it supports growth of many enteroviruses, including polioviruses), and L20B cell line (genetically modified mouse L cells expressing the human poliovirus receptor (CD155); highly selective for poliovirus isolation and identification. Isolates were subjected for real-time RT–PCR, whereas cultures were checked for cytopathic effect. Negative for poliovirus but positive in RD cells were classified as NPEVs (2, 14, 15). Laboratory work was performed in biosafety cabinets, incubated at 37°C/5%CO_2, centrifuged and tested using a real-time PCR system under WHO quality assurance.

Data were analyzed utilizing IBM SPSS Statistics. Categorical variables were presented as frequencies and percentages, and compared using chi-square or Fisher's exact tests. The Binomial distribution (with 95% confidence intervals) will be used to describe the epidemiological profile of NPEV cases among NPEV AFP cases in Iraq, for the period 2018–2024. Statistical significance was depicted by a p-value <0.05.

Ethical approval and data access permissions from national authorities were also obtained. These data were unidentified before the analysis was started.

Results

The data set is a time series of AFP cases that are NPEV positive during 2018-2024. The percentage of AFP cases who were positive for NPEVs varied from 12.4% to 13.9% during the period 2018–2019. This was at 9.2% in 2020 and was low between 2021-2023, ranging from 7.1% to 8.4%. The percentage of NPEV-positive cases increased to 12.0% in 2024. Over the study period, NPEVs were present in 10.5% of AFP cases (594/5662 samples), with the proportion of NPEV-negative remaining consistently high (86.1–92.9%) as indicated in Table 1.

Table 1: Distribution of cases with AFP by Year

Year	NPEV +VE		NPEV –VE		Total
	No.	Percent	No.	Percent	
2018	127	12.4%	896	87.6%	1023
2019	161	13.9%	996	86.1%	1157
2020	44	9.2%	432	90.8%	476
2021	50	7.1%	659	92.9%	709
2022	70	8.4%	765	91.6%	835
2023	65	7.9%	755	92.1%	820
2024	77	12.0%	565	88.0%	642
Total	594	10.5%	5068	89.5%	5662

The results of 3,341 contact samples were examined; among these the percentage of NPEVs-positive was found to be 9.6% (321 cases) and that of negative as 90.4% (n = 3020). The highest positivity recorded was 93 (11.6%) cases in 2018, followed by steep decrease in the subsequent years- (6.5% each during 2019 & 2020) amidst healthcare system disturbances owing to the COVID-19 pandemic. Positivity restored in 2021, amounting to 46 cases with percentage of (10.1%) by 2024 Table 2.

Table 2: Distribution of contacts by years

Year	NPEV +VE		NPEV -VE		Total
	No.	Percent	No.	Percent	
2018	93	11.6 %	711	88.4 %	804
2019	39	6.5 %	561	93.5 %	600
2020	11	6.5 %	157	93.5 %	168
2021	29	9.2 %	285	90.8%	314
2022	47	9.6 %	445	90.4%	492
2023	56	11.0%	453	89.0%	509
2024	46	10.1 %	408	89.9%	454
Total	321	9.6%	3020	90.4%	3341

Distribution of NPEV-positive AFP cases by year shows distinct seasonality. There appear to be two peaks; one in January (62 cases) and another in May (61cases). Cases are particularly low in September, where a minimum of 31 cases is recorded. Mid-year (May to July) and early months (January to March) always report more cases, with August and September being a dip in activity as can be seen in Figure 1.

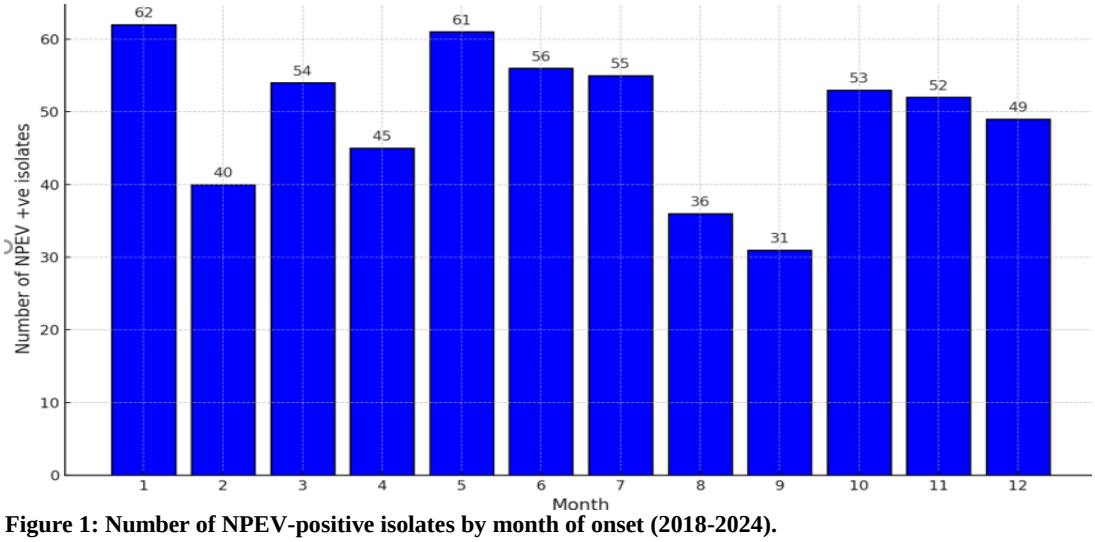

Figure 1: Number of NPEV-positive isolates by month of onset (2018-2024).

Gender-based analysis showed that out of 3,301 males with AFP; 358 (10.8%) were NPEV positive, and among 2,361 females with AFP; 236 (10.0%) were NPEV positive . The difference was statistically not significant -value =0.30) Table 3.

Table 3: Distribution of NPEV cases by gender

Gender	NPEV +VE		NPEV -VE		Total
	No.	Percent	No.	Percent	
Male	358	10.8%	2943	89.2%	3301
Female	236	10.0%	2125	90.0%	2361
Total	594	10.5%	5068	89.5%	5662

The ages can be divided into 5 age groups: ≤1 year , >1-2 year , >2-3 year , >3-4 years and >4years old. It presented the maximum of frequency value 169 (14.5%) cases of NPEV positivity among children between1–2 years, followed by 71(11.7 %) cases in under one year age . The lowest positivity 195(7.6%) was found among children (4-15years). The prevalence of NPEVs-negative also showed age correlation and younger children were more likely to have NPEV positive. There is a significant association between age and NPEV positivity ($p < 0.001$), with younger children being affected more often than older children as presented in the Table 4.

Table 4: Distribution of AFP cases by age

Age (year)	NPEV +VE		NPEV -VE	Total	
	No.	Percent	No.	Percent	
< 1	71	11.7%	538	88.3%	609
>1 – 2	169	14.5%	995	85.5%	1164
>2 – 3	94	12.5%	658	87.5%	752
>3 – 4	65	11.4%	506	88.6%	571
>4 – 15	195	7.6%	2371	92.4%	2566
Total	594	10.5%	5068	89.5%	5662

Of the patients who tested NPEV positive; 412 (10.1%) cases presented with fever while 143 (10.9%) without fever. There was no significant association between NPEV positivity and fever (p=0.76). There was higher NPEV positivity in patients with asymmetrical weakness 183 cases with (11.5%) compared to those of symmetrical weakness 408 with (10.0%). However, there was no significant association (p=0.10).

Patients with residual weakness were more likely to have NPEV positivity [(72 cases; 14.2%) than those without residual weakness (501 cases 10.4%)]. There was a significant association between residual weakness and NPEV positivity (p=0.007). Mortality data showed less NPEV positivity among deceased 4 (3.4%) cases compared to survivors 573 (10.7%) cases. This difference was statistically significant (p= 0.009) as presented in Table 5.

Table 5: Association of clinical characteristics with NPEV Positivity

Clinical Characteristic	NPEV +ve		NPEV –ve		Total		P-value
	No.	Percent	No.	Percent	No.	Percent	
Fever	412	74.2%	3643	37.6%	4055	73.7%	0.76
No fever	143	25.8%	1304	26.4%	1447	26.3%	
Asymmetrical	183	33.5%	1399	27.8%	1582	28.1%	0.10
Symmetrical	408	66.5%	3632	72.2%	4040	71.9%	
Residual weakness	72	12.5%	434	9.1%	506	0.95 %	0.007
No residual weakness	501	87.5%	4328	90.9%	4829	90.5 %	
Died	4	0.06%	115	2.3%	119	2.2 %	0.009
Survived	573	99.4%	4780	97.7%	5353	97.8 %	

Discussion

Surveillance for AFP is one of the key public health interventions, which helps to monitor poliovirus eradication and manage non-polio etiologies, such as, NPEVs. This study highlights the significant patterns in AFP cases and their corresponding clinical and epidemiological features. A decline in AFP cases, during 2020, reflects the disarray in global healthcare services due to the COVID-19 pandemic (16,17).
These interruptions, which have been documented globally, highlight the gaps in surveillance systems during crises. Yet, the subsequent rebound in AFP case detection demonstrates that public health systems can recover and adapt to adversity (16). The NPEV detection rate (10.5%) in our study aligns with findings from other regions, highlighting the contribution of NPEVs as a major cause of AFP in the polio eradication era (3,4). The NPEV positivity among AFP contacts was 9.6%, which was lower when compared to the index cases, as seen in other studies (17,18). Reduced surveillance, in 2020, has provided an additional example of the deleterious effect of global crises on public health systems (19). Strengthening case-based and environmental

surveillance is essential to close the possible immunity gaps and avoid the reintroduction of poliomyelitis (20).

The seasonal pattern in NPEV-positive AFP cases reflect that of Enteroviruses, seen worldwide. The January peaks may reflect the influence of human dynamics. The January social activities have increased above the December activities, but the May peaks indicate increased stability among viruses in that season. Seasonal patterns suggest that AFP surveillance should be conducted throughout the year, to capture these changes. (8,21,22).

There was no significant difference between the positivity rates in male and female subjects (p= 0.30), which was consistent with other reports. These reports stated that exposure risk did not differ by gender. However, the higher number of cases in males may be due to behavior and cultural factors, such as spending more time outdoors or seeking medical care more often (6,23).

The age at which children had the highest NPEV positivity was one to two years (14.5%), reflecting that exposure to these infections may be more common because of their less developed immune systems and increased contact from fecal-oral transmission pathogen routes, including ready-to-eat food preparations. This was also noted in a previous study in Iraq on NPEV and demographic characteristics for the period from 2010-2017 using the same age group distribution (23). This might suggest the necessity of interventions in the younger age groups, in the form of vaccination and hygiene education.

The age distribution particular to our study was in line with data from AFP-based surveillance performed in countries around Iraq. In Turkey, the peak prevalence of NPEV detection was in children aged 1–4 years (around 44.5%) with lower rates in older age groups (24). Data from Pakistan also showed that 84% of NPEV isolate in AFP cases occur in children <5 years of age, and NPEV detection decreased significantly with increasing age. As a result, it was likely to reflect acquired immunity (25).

All of these results together confirm that this pattern of NPEV infection is largely limited to young children within the region, consistent with the idea that high levels of fecal–oral transmission led to rapid early exposure followed by a period of immunity acquisition and limited virus detection as age increases. In developed countries, AFP-based data show overall lower NPEV positivity and a more diverse age distribution than in developing countries. Importantly, NPEV detection in AFP cases was only 4.7% in Greece (versus an average of 25–30%) in Low- and Middle-Income Countries (6). These observations imply that in developed settings improved sanitation and less fecal–oral transmission delay initial exposure, shifting NPEV infections to older ages with reduced overall positivity; paradoxically, early-life exposure is associated with a higher positivity in developing settings. (26). Fever, although observed frequently in viral infections, was not significantly associated with being NPEV positive, and hence, might be an unreliable sign of NPEV infection. The symmetrical quality of the weakness was not significantly related to NPEV positivity. Symmetric and asymmetric patterns of weakness were both reported, underscoring the broad clinical spectrum determined by the host-virus interactions (27). Residual weakness was highly associated with NPEV positivity, which suggested lasting neurological complications. This finding aligned well with other researches on Enteroviruses D68, which have a reputation for inflicting long-term neurological deficits. Early diagnosis and treatment are key to the impact of these influences (28).

Mortality in AFP cases also accentuates the complexity of NPEV positivity trends. The lower fatality rate in NPEV-positive cases of AFP may suggest a relatively benign disease course, as compared to the NPEV- negative cases of AFP, which have a high mortality rate. The latter may be due to other etiologies, co-morbidities, or possibly delays in case handling (23).

Limitations

The NPEV isolates were not serotyped or genetically defined and therefore precluded an assessment of strain-specific epidemiology. The analyses were confined to AFP cases and therefore may not be representative of NPEV circulation in the general population. Furthermore, the COVID-19 pandemic may have impacted surveillance activities which probably interfered with case detection.

Conclusion

During 2018–2024; NPEV had contributed to 10.5% of all AFP cases with a changing trend that started with an increase until 2019 and then decreased during the periods of 2020–2023 before showing its recovery in years of 2024. A similar temporal pattern in contacts was probably due to COVID-19 surveillance again. NPEV positivity showed a distinct age-dependent pattern, with higher rates in younger children especially those aged 1–2 years and much lower rates in older children. There was a significant seasonal variation with its peaks in early and mid-year months. There were no significant associations with gender, fever or pattern of weakness; but NPEV positivity was significantly associated with residual weakness on exam and lower in fatal cases.

Authors' declaration

We confirm that all the Figures and Tables in the manuscript belong to the current study. Besides, the figures and images, which do not belong to the current study, have been given permission for re-publication attached to the manuscript. Authors sign on ethical considerations. Approval-Ethical Clearance: The project was approved by the local ethical committee (Department of Family and Community Medicine, College of Medicine,

University of Baghdad.) according to the code number (268) on (17/12/ 2025).

Informed Consent

Patient consent was waived because the study involved retrospective review of unidentified medical records, with no direct patient contact.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Data availability

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

AI Declaration

No artificial intelligence tools were used in the design, analysis, or writing of this manuscript.

Authors' contributions

Study conception & design: (Nadia A. Nasir & Murad Th. Mehmoud). Literature search: (Muhi K. Al-Janabi).
Data acquisition: (Murad Th. Mehmoud).
Data analysis & interpretation: (Mohanad H. Naqqash & Husam K. Ayoob).
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الفايروسات المعوية غير المتعلقة بشلل الاطفال في الشلل الرخوي الحاد: تحليل الرصد في العراق من 2018 إلى 2024

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الخلاصة:

الخلفية: يشكل رصد الشلل الرخو الحاد الأساس لجهود استئصال فيروس شلل الأطفال ويوفر أيضًا بيانات مفيدة عن الفيروسات المعوية غير المتعلقة بشلل الأطفال والتي تعد من الأسباب المهمة للإصابة بالشلل الرخو الحاد في البلدان الخالية من شلل الأطفال. بعد إعادة استيراد فيروس شلل الأطفال البري في عام 2014 وعلى الرغم من شهادة خلو المنطقة من شلل الأطفال في عام 2015، مما أدى إلى تعزيز مكثف لمراقبة الشلل الرخو الحاد. بحلول الفترة 2018-2024، حافظ العراق على خلوه من شلل الأطفال مع تحقيق مؤشرات المراقبة لمنظمة الصحة العالمية بشكل متسق، بما في ذلك معدلات الشلل الرخو الحاد غير المتعلقة بشلل الأطفال وجودة عينات البراز. خلال هذه الفترة، أصبحت الفيروسات المعوية غير المتعلقة بشلل الأطفال هي المعزولات السائدة بين حالات الشلل الرخو الحاد، مما يعكس حساسية المراقبة الفعالة واستمرار تداول الفيروس المعوي.

الأهداف: تهدف هذه الدراسة إلى تحديد مدى انتشار واتجاهات الوبائية والسمات السريرية للفيروسات المعوية غير المتعلقة بشلل الأطفال بين حالات حالات الشلل الرخوي الحاد لدى الأطفال العراقيين الذين تقل أعمارهم عن 15 عامًا، بين عامي 2018 و 2024، لتحسين حساسية المراقبة وتدابير الصحة العامة التكميلية القائمة على الأدلة.

الطرق: تم جمع البيانات من نظام المراقبة في العراق. تم تحليل عينات البراز في المختبر الوطني لشلل الأطفال وفقاً لإرشادات منظمة الصحة العالمية. تم تصنيف الحالات التي كانت سلبية بالنسبة لفيروس شلل الأطفال، ولكن كان لها تأثير اعتلال خلوي على خلايا الساركوما العضلية المخططة على أنها الفيروسات المعوية غير المتعلقة بشلل الأطفال وقد تم تحليل التركيبة الديموغرافية والنتائج السريرية إحصائيا.

النتائج: في 5662 حالة من حالات الشلل الرخو الحاد، تم الإبلاغ عن الفيروسات المعوية غير المتعلقة بشلل الأطفال في 10.5% من الحالات؛ يكون معدل الانتشار أعلى عند الأطفال من سنة إلى سنتين (14.5%). ولوحظ انخفاض في اكتشاف الفيروسات المعوية غير المتعلقة بشلل الأطفال خلال جائحة كوفيد-19، لكنه عاد بحلول عام 2024. وارتبطت الفيروسات المعوية غير المرتبطة بشلل الأطفال بالضعف المتبقي (14.2%)، وانخفاض معدل الوفيات (3.4%).

الاستنتاجات: أظهر اكتشاف الفيروسات المعوية غير المتعلقة بشلل الأطفال انخفاضًا خلال الفترة 2020-2023 مع التعافي في عام 2024، بالتوازي مع الملامسين. وكانت الإيجابية أعلى بشكل ملحوظ لدى الأطفال الأصغر سنا، وخاصة 1-2 سنة، وأظهرت زيادة موسمية واضحة. ارتبطت عدوى الفيروسات المعوية غير المتعلقة بشلل الأطفال بالضعف المتبقي ولكن ليس بالجنس أو الحمى أو الوفيات.

• **الكلمات المفتاحية**: الفيروسات المعوية غير المتعلقة بشلل الأطفال، الشلل الرخو الحاد، المراقبة، الخصائص الديموغرافية، الضعف المتبقي، الوفيات، العراق.