ORIGINAL ARTICLE

Non-polio Enterovirus Isolation in children with acute Flaccid Paralysis in Iraq

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ABSTRACT

Aim: This study is an overview of NPEV investigated during AFP surveillance programs for the period 2010–2017 in Iraq.
Methods: Stool samples from 4296 AFP cases and 2933 healthy contacts among children less than 15 years of age were processed for virus isolation as a part of AFP surveillance for the Global Polio Eradication Program in Iraq at National Polio Laboratory. NPEV detection was performed by virus isolation on cell culture according to WHO recommendations.
Results: The NPEV isolation rate was 14% of total AFP cases and 14.5% of healthy contacts. The infection rate was higher in males than females with a male/female ratio of 1.5:1. The highest NPEV infection rate was observed among the children aged 1-2 years and decrease significantly with increasing age. Residual weakness after 60 days from paralysis onset was reported in 26% and death in 1.5% of AFP cases with positive NPEV isolates.
Conclusion: The NPEV circulation is common in our environment and may play a role in causing AFP cases especially for younger age groups, NPEV could be isolated from healthy persons and from persons whose clinical findings do not resemble poliomyelitis.
Keywords: Non-polio, enterovirus, acute flaccid paralysis

INTRODUCTION

Since the launch of the Global Polio Eradication Initiative in 1988, AFP surveillance had been conducted as a part of WHO strategies for polio eradication. AFP constitutes sudden onset of weakness and floppiness in any part of the body of a child less than 15 years of age, or paralysis in any person with suspected poliovirus infection.(1) In Iraq, National Polio Lab. (NPL) was accredited by WHO, assisting the program by processing stool specimens to identify positive cultures for both Polioviruses and NPEV for clinical syndromes of AFP and their contacts. (2,3) Enteroviruses (EVs) belonging to the family Picornaviridae are common human pathogens. EV infection is usually asymptomatic but sometimes associated with diverse clinical symptoms ranging from minor febrile illness to severe potentially fatal aseptic meningitis, viral encephalitis, paralysis, myocarditis and neonatal sepsis.(4) NPL investigates stool samples by using special cell cultures specified for Polio Viruses (PVs) and NPEVs. Besides PVs, a considerable proportion of NPEVs can be isolated from AFP surveillance as well. All AFP cases are followed for 60 days after paralysis onset to verify the presence or absence of residual weakness or patient death. (5) The study describes the epidemiology, clinical presentation of AFP cases with NPEV isolates, and to know the sequelae of NPEV on the child wellbeing sixty days after paralysis onset, that is to gain a better understanding of the potential importance of NPEV isolation for poliomyelitis eradication program.

MATERIALS AND METHODS

By using the national federal AFP surveillance system database (IFA), all reported AFP cases for children below 15 years with onset of paralysis in the period 2010-2017 were identified. As a notifiable disease AFP cases were reported on a weekly base to the central level.

Contact stool samples are collected from children who have the closest contact with the hot AFP cases, inadequate AFP cases and clusters of AFP cases. All stool specimens from AFP cases and contacts were tested in an accredited national lab. (NPL) for viral isolation. Stool samples are processed according to the WHO protocols. They are inoculated in human rhabdomyosarcoma (RD) and recombinant murine (L20B) cell lines. The stool specimens producing a cytopathic effect (CPE) in RD cells are passed on to L20B cells, if CPE is not produced, then they are considered as NPEVs (5, 6, 7). IFA data management for surveillance of AFP cases was used in data analyses. (Epi-C version 3.1.2).

RESULTS

A total of 4296 AFP cases in children less than 15 years were reported during the period 2010-2017. NPEVs were isolated in 602/4296 (14%) of total AFP cases. The NPEV isolation was variable over the study years ranging from 7.3% to 18.2% suggesting a relatively high prevalence (Table-1). NPEVs were isolated in 421/2933 (14.5%) of healthy contacts (Table -2). The infection rate was higher in males than females (60.3% were males and 39.7% were females) with a male/female ratio of 1.5:1 (Table-3). The highest NPEV infection rate was observed among the children aged >1-2 years (28.5%) and decreased significantly with increasing age, P: < 0.001 (Table-3). NPEVs positive AFP cases were detected throughout the year with some seasonal variation. A high period of transmission was observed during spring and autumn peaking in May and November (70 and 73 cases respectively) with a nadir of 28 cases in August (Figure-1). Children with positive NPEVs isolates presented with fever and AFP status were 133/602 (22%) and those with asymmetrical

paralysis were 198/206(33%). The outcome after 60 days follow up since the date of paralysis onset of AFP cases with NPEV isolates showed residual weakness in 158/602(26%) and death in 9/602(1.5%), while 16 (2.6%) were lost to follow up as seen in Table-4.

Table 1: Frequency of children with NPEV isolates among AFP cases (2010-2017)

Year of onset	+ve NPEV No. (%)	-ve NPEV No. (%)	Total AFP cases
2010		392 (81.8)	479
2011	94 (17.9)	431 (82.1)	525
2012	77 (16.5)	389 (83.5)	466
2013	32 (7.3)	406 (92.7)	438
2014	83 (14.2)	502 (85.8)	585
2015	76 (14.8)	439 (85.2)	515
2016	61 (10.2)	535 (89.8)	596
2017	92 (13.3)	600 (86.7)	692
Total	602 (14.0)	3694(86.0)	4296

Table 2: Frequency of children with NPEV positive isolates among healthy contacts 2010-2017)

Year of onset	+ve NPEV No. (%)	-ve NPEV No. (%)	Total contacts
2010	31 (20.3)	122 (79.7)	153
2011	23 (14.9)	131 (85.1)	154
2012	16 (11.7)	121 (88.3)	137
2013	19 (10.4)	164 (89.6)	183
2014	29 (12.0)	212 (88)	241
2015	105 (15.5)	571 (84.5)	676
2016	101 (14.3)	604 (85.7)	705
2017	97 (14.2)	587 (85.8)	684
Total	421 (14.4)	2512 (85.6)	2933

Table 3: The association between demographic characteristics and NPEV isolation in AFP cases (2010-2017)

Characteristics	+ve NPEV No. (%)	-ve NPEV No. (%)	P value
Gender			
Male	363 (60.3)	2206 (59.7)	0.19
Female	239 (37.7)	1488 (40.3)	
Total	602 (100)	3694 (100)	
Age			
1 mo.-1 year	80 (13.2)	387 (10.5)	<0.001
>1-2 year	171 (28.5)	791 (21.4)	
>2-3 year	144 (23.9)	606 (16.4)	
>3-4 year	112 (18.6)	780 (21.1)	
>4-15 year	95 (15.8)	1130 (30.6)	

Figure 1: Monthly distribution of NPEV isolates in AFP cases

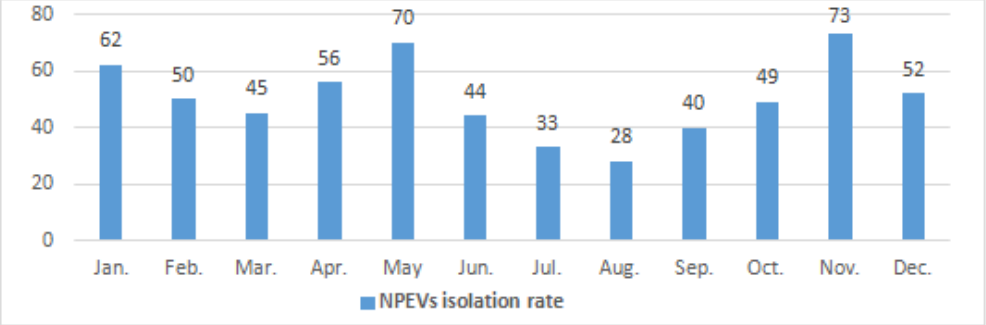
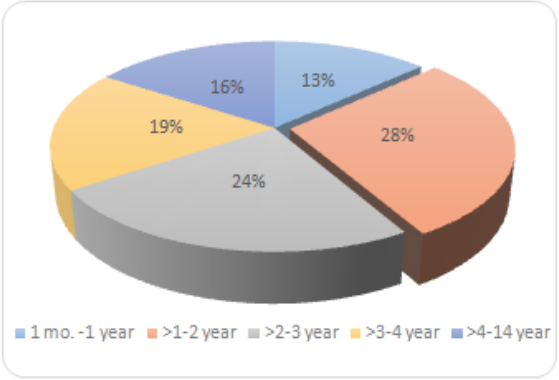


Figure 3: Age groups of children with NPEV positive isolates among AFP cases



Year of onset	Total	fever	Asymmetrical
2010	87	22 (25.3)	33 (37.9)
2011	94	21 (22.3)	32 (34.0)
2012	77	13 (16.9)	23 (29.9)
2013	32	3 (9.4)	5 (15.6)
2014	83	23 (27.7)	39 (47.0)
2015	76	15 (22.4)	21 (2.8)
2016	61	11 (18.0)	13 (21.3)
2017	92	25 (27.2)	32 (34.8)
Total	602	133 (22%)	198 (33%)

Table 4: Clinical presentations of children with NPEV positive isolates among AF cases

Table 5: Clinical presentations and outcome after 60 days follow up of children with NPEV isolates among AFP cases

Year of Onset	Fever	Asymmetrical	Residual Weakness	No. of Residual weakness	Died	Lost of follow up	Total
2010	22	33	21	60	2	4	87
2011	21	32	29	61	2	2	94

2012	13	23	23	52	1	1	77
2013	3	5	13	17	0	2	32
2014	23	39	21	57	0	5	83
2015	15	21	18	54	3	1	76
2016	11	13	14	47	0	0	61
2017	25	32	19	71	1	1	92
Total	133(22%)	198(33%)	158(26%)	419 (69.6%)	9 (1.5%)	16 (2.6%)	602(100%)

DISCUSSION

Over the period of 2010-2017; 602/ 4296 (14%) of AFP cases were positive for NPEV at Iraqi NPL. The WHO suggests that, at least, 10% of all fecal specimens received into the reference laboratory should be positive regarding non-polio enterovirus isolation. This indicator points out the laboratory ability to perform enterovirus isolation[7]. It is not possible to determine whether such isolates were directly related to the paralysis or represent background Enterovirus circulation. [(8)] To have a sensitive detection of poliovirus in hot AFP cases, inadequate stool specimens and clusters of AFP cases, stool collection is taken from children in closest contact with the index case.

All cases with high suspicion of polio including young age group, asymmetrical paralysis with fever, rapid progression of paralysis, and partially or not vaccinated are labelled as hot case). [(3,9)] Therefore in addition to AFP cases, their healthy close contacts were surveyed for NPEV and the result showed 14.5% of contacts were positive for NPEV isolates. The NPEVs isolated positive results of the contacts may indicate that children were infected without being pathogenized with these viruses i.e. subclinical infections or asymptomatic carriers of virus. In addition, it may represent a threat in this group to induce a clinical AFP picture. [(10)] The result of this study is close to a previous study conducted in Iraq in 2015 by Mays et al[8] demonstrated NPEV in a rate of 14.3% of AFP cases. It is higher than those in Pakistan (8.5%), Brazil (6.7%) and Tunisia (5.3%). However, it is lower than those in Egypt (17.6%) and Ghana (20%). [(7)] Lower isolation rates may be explained by the fact that many NPEV strains belonging to the Enterovirus C species cannot be isolated on RD cells. Moreover, studies showed that the introduction of HEp-2c cell line in the isolation process increases the NPEV isolation rate[7, 11, 12].

The isolation rates below 10% may reflect a cold chain problem in keeping and transportation of the stool sample. Other factors may explain the decrease in the NPEV isolation rate like low sensitivity of the isolation methods or inadequate sample collection and storage before the transportation. Therefore, NPEV rate may no longer a strict criterion for accreditation of reference laboratories as isolation rate is affected by other factors such as season, altitude, and population hygiene. However, the rate may be a useful indicator of laboratory performance. [(10)] In our study, the male/female ratio is 1.5:1 among AFP children with NPEV isolates. The male predominance explanation goes for some Iraqi families who take care of male patients more than females but this is not true for all communities in Iraq. Male predominance among AFP cases with positive NPEV isolates was also reported in other countries[9, 11.]

Age distribution shows more than 84% were under 5 years, which is harmonized with the overall AFP Federal data[(11] These findings were approved by Mays et al [(8)]. A higher isolation rate was reported by V. Dietz et al. in USA where 54.3% were above 5 years of age[9]. Our findings show fever in 22%, asymmetrical paralysis in 33% of the cases with positive NPEV isolates. This, in turn, makes these cases likely to be confused with poliomyelitis cases. Regarding the outcome of AFP cases with positive NPEV isolates; our data show that 26% had residual weakness after 60 days from paralysis onset, 1.5% died and 2.6% lost to follow up. Those who died were nine children. Eight of them were diagnosed as Guillain-Barré syndrome and the one was diagnosed as bacterial meningitis.

It should be noted that these findings were obtained from an ongoing AFP surveillance system that was not designed to evaluate NPEV as a cause of AFP since it focused on poliomyelitis and polioviruses; on the other hand, NPEV typing was not performed at NPL at the time of data collection.

CONCLUSION

This study confirms that NPEV circulation is common in our environment (among AFP cases and healthy contacts) and may play a role in causing acute flaccid paralysis especially for younger age groups, however, small percentages might develop fever with asymmetrical paralysis, which may end with residual weakness after 60 days follow up examination. On the other hand, NPEV could be isolated from healthy persons and from persons whose clinical findings do not resemble poliomyelitis. The results of this study could provide a better understanding of the epidemiology of paralysis caused by NPEV and potentially contribute to the establishment of environmental surveillance and assessing the role of NPEVs serotypes in children with AFP.

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