

Research Article

Evaluation of the Acute Flaccid Paralysis Virological Surveillance System in Polio-Free Morocco, 2010–2018

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Background. The goal of Acute flaccid paralysis (AFP) surveillance is to comprehensively investigate and report any case of a child below 15 years with a floppy weakness. This is essential for the poliovirus eradication initiative. **Objective.** In this study, we analyzed the results of nine-year surveillance (2010–2018) of AFP in Morocco. **Method.** This was a descriptive, retrospective study of cases with AFP routinely documented at the National Referral Laboratory of Polio (NRLP) from 2010 to 2018. **Results.** Among the 884 AFP cases identified and analyzed, we identified 11 polioviruses as Sabin-like vaccine strains (PSL). PSL were isolated in 11 samples of AFP cases received at the laboratory, including one PSL1, three PSL2 + PSL3, one PSL1 + PSL2, and one PSL1 + 2 + 3. The annualized nonpolio AFP rate per 100,000 children under 15 years ranged from 0.84 during 2011–2015 to 2.29 in 2018. The stool adequacy indicator was poor and may be improved with better delivery times and maintaining the cold chain. The incidence of isolated nonpolio enteroviruses (NPEV) varied between 2.27% and 12.6%, with the average not exceeding 6.35%; the incidence remained low compared to the indicator set by the World Health Organization (WHO). **Conclusion.** Morocco was certified polio-free by WHO in 2015, and since then no cases of wild poliovirus have been reported. Overall, although the nonpolio AFP rate has risen to the WHO standard, stool adequacy and the proportion of nonpolio enteroviruses need to be improved. The laboratory can adopt alternative techniques, independent of cell culture, to ensure that imported cases of poliovirus are not missed, especially in this era of eradication.

1. Introduction

Poliomyelitis is an infectious viral disease that mainly affects children below five years. Less than 1% of children infected with polio develop paralysis, and for every child with paralysis, there are approximately 200 children who are infected but asymptomatic [1]. There is no cure for polio; however, it can be prevented through immunization. The widespread use

of oral polio vaccine (OPV), containing live attenuated poliovirus types 1, 2, and 3, has been the primary method to prevent polio since the early 1960s. However, the attenuated polioviruses contained in OPV can undergo genetic changes during replication and, in communities with low immunization coverage, can give rise to vaccine-derived polioviruses (VDPVs) that can cause paralytic polio similar to the disease caused by wild polioviruses (WPV) [2].

The Global Polio Eradication Initiative (GPEI) was launched by the World Health Organization (WHO) in 1988. Since then, cases of WPV have decreased by approximately 99.99%, and WPV2 and WPV3 types were declared eradicated. Transmission of WPV type 1 (WPV1) is still uninterrupted only in Afghanistan and Pakistan [3]. The key strategies in the GPEI include (1) strengthening childhood immunization through oral polio vaccines, (2) conducting surveillance through investigation of AFP cases among children below 15 years, and (3) conducting house-to-house “mop-up” campaigns in areas where cases of polio have been detected [4]. To achieve a “certification standard,” the AFP surveillance systems need to detect at least one case of nonpolio AFP per 100,000 children below 15 years every year and collect two adequate stool specimens from at least 80% of cases with AFP, with the stool specimens tested for poliovirus at a WHO-accredited laboratory [5]. Morocco implemented this GPEI based on the following strategies recommended by the WHO: routine immunization of infants with at least three doses of the oral polio vaccine (OPV), annual national immunization days, during which two additional doses of OPV are administered to all children under five years regardless of their previous immune status, and surveillance of all cases of AFP in a virological laboratory. Since 1990, no case of poliomyelitis has been reported in Morocco. The average vaccination coverage of the population is about 95% with the use of the oral polio vaccine (OPV) and the introduction of the inactive polio vaccine in 2015 [6].

The National Referral Laboratory of Polio (NRLP) has strongly promoted the polio control program and achieved its objectives by ensuring the isolation and identification of the strains of polioviruses causing paralysis. The laboratory was accredited by the WHO in 2001. The accreditation is annually granted after the validation of a performance test or “proficiency testing” through sample analysis and a site visit by a WHO consultant, who evaluates the compliance standards of the laboratory premises and platforms, as well as, the technical procedures followed [7].

The NRLP receives stool samples of all cases of AFP reported in Morocco. Two stool samples of adequate quantity (~8 g) are collected at least 24 h apart from each child with AFP and below 15 years within 14 days of the onset of paralysis. If the sample is collected after 14 days of the onset of paralysis, a stool sample is also collected from five children below 15 years who have been in contact with the child with AFP. The NRLP ensures the isolation and identification of poliovirus in samples collected locally and sent by the surveillance officers. Owing to the efforts of all the stakeholders in the Ministry of Health, Morocco was declared a “Polio Free” zone by the WHO in 2015.

In this study, we analyzed the results of nine-year surveillance (2010–2018) of AFP in Morocco and identified the weak areas that need to be improved to meet the WHO requirements under the GPEI.

1.1. Ethics Statement. This study was a part of the GPEI. Fecal samples from children with AFP were sent to the

laboratory for isolation of poliovirus by cell culture. The technical and ethical protocols were validated by the WHO and the Ministry of Health of Morocco.

1.2. Study Area. The kingdom of Morocco is a country in North Africa bordering the Atlantic Ocean and the Mediterranean Sea. Morocco has 12 administrative regions [8]. The population of Morocco was 36,670,141 on 14 December 2019, based on the Worldometers report of the latest data from the United Nations [9].

2. Methodology

2.1. Study Design. This was a descriptive, retrospective analysis of the stool samples of AFP cases routinely collected at the NRLP of the National Institute of Hygiene between 2010 and 2018.

2.2. Patients and Samples. During the study, 2529 stool samples were received at the NRLP between 2010 and 2018. Of these, 1799 stool samples were received from 884 AFP cases, and 730 were samples of the contacts of children with AFP (Table 1).

2.3. Specimen Preparation, Virus Isolation, and Characterization. The specimens were pretreated before inoculation in the culture medium, and all fecal samples were treated with chloroform. Besides removing the bacteria and fungi, this method also removed potentially cytotoxic lipids and dissociated the virus aggregates [10]. The WHO specified that fecal suspension from all AFP cases should be inoculated in cell cultures of RD (human rhabdomyosarcoma) and L20B (mouse L cells transfected with human cellular receptor gene for poliovirus) and incubated in a stationary inclined position at 36°C. The isolates that showed a cytopathic effect (CPE) only in the RD cell line were assumed to be nonpolio enteroviruses (NPEV), and those that showed CPE on both cell lines, i.e., RD and L20B, were considered to be polioviruses and subsequently subjected to intratypic differentiation (ITD) with a real-time reverse transcriptase polymerase chain reaction (Poliovirus rRT-PCR ITD 5.0 and Poliovirus rRT-PCR VDPV 5.0 kits, Centers for Disease Control, Atlanta, GA, USA). The ITD/VDPV assay was performed to classify polioviruses as nonenterovirus (NE), nonpolio enterovirus (NPEV), vaccine-like (Sabin-like PSL) poliovirus type 1, 2, or 3, or vaccine-derived poliovirus (VDPV) [7].

3. Results

3.1. Demographic and Geographic Characteristics of AFP Cases. Of the 978 AFP patients identified and analyzed, 57.06% were below five years, and 56.65% were male (Table 2). The vaccination status of 62% of these children was known.

An analysis of the notification of AFP cases per region showed high notification in the Fes-Meknes region, followed by the Casablanca region; these are the northern and eastern

TABLE 2: Characteristics of AFP cases reported in Morocco between 2010 and 2018.

Characteristic	Value, n (%)
Sex	
Male	554/978 (56.65%)
Female	424/978 (43.35%)
Age (years)	
Unknown	40/978 (4.09%)
<1	53/978 (5.42%)
1–5	505/978 (51.64%)
6–10	228/978 (23.31%)
11–15	152/978 (15.54%)

TABLE 3: Nonpolio AFP rate, percentage of AFP cases with two adequate stool specimens and NPEV rate (WHO).

Target		Year								
		2010	2011	2012	2013	2014	2015	2016	2017	2018
Nonpolio AFP rate per 100,000 children under 15 years	≥1	1.1	0.84	0.89	1.16	1.12	0.84	0.9	1.52	2.29
% Adequate stool collection	≥80	78	80	81	70	71	57	61	67	78
NPEV rate	>10%	6.80% (11/161)	12.60% (16/126)	2.27% (3/132)	6.35% (11/173)	6.28% (11/175)	5.51% (8/145)	8.82% (15/170)	5.98% (14/234)	2.54% (10/393)

regions of Morocco, respectively (Table 1). Very few cases were reported from Guelmim-Oued Noun and Laayoune-Sakia El Hamra, while no cases were notified in the Dakhla-Oued Ed Dahab region.

3.2. Nonpolio AFP Rate. At least one case of nonpolio AFP should be reported each year for every 100,000 children below 15 years. The average nonpolio AFP rate during the nine-year surveillance was 1.18. It ranged from 0.84 in 2011–2015 to 2.29 in 2018 (Table 3).

3.3. Stool Adequacy. The WHO stipulates that two stool samples must be collected at least 24 h apart, both within 14 days of the onset of paralysis, transported under cold storage with ice, and received in good condition at the WHO-accredited laboratory. At least 80% of the cases must have two adequate stool samples. The adequate stool collection rate ranged from 81% (in 2012) to 57% (in 2015). Three periods were described regarding stool adequacy. The first period corresponded to acceptable stool conditions (2011–2012). During the second period, the rate decreased to 70% in 2013 and further decreased to 61% in 2016. In the third period, the stool adequacy rate steadily increased to 67% in 2017 and 78% in 2018 but was lower than the recommended rate of at least 80% (Table 3).

3.4. Polioviruses Isolated from Stool Specimens. In total, 11 polioviruses were identified by ITD as Sabin-like vaccine strains (PSL). PSL were isolated from 11 samples of AFP cases received at the laboratory. One case was of PSL1, three cases each of PSL2 and PSL3, one case was of combined PSL1 and PSL2, and one case was of combined three vaccine strains 1,2, and 3 (PSL1 + 2 + 3).

3.5. NPEV Isolated from Stool Specimens. NPEV should be isolated in at least 10% of stool specimens submitted to the laboratory. It is an indicator of the quality of the reverse cold chain and also the efficiency of the laboratory in performing routine isolation of the enterovirus. In total, 99 NPEV were isolated by cell culture from the stool samples of AFP cases. The incidence of isolated NPEV was calculated based on the number of samples of AFP cases and their contacts. This incidence varied from 2.27% to 12.6% (Table 3), and the average did not exceed 6.35%; the incidence remained low compared to the indicator set by the WHO.

The group that was most affected by the NPEV infection included children under five years, corresponding to 87% of the AFP cases (86/99 patients).

3.6. Turnaround Times for Reports of AFP Cases to the WHO. The three standard indicators of laboratory timeliness for processing the stool specimen included reporting ≥80% isolation results within 14 days of sample receipt, reporting >80% ITD results within seven days of receiving the sample, and shipping ≥80% of WPV and suspected VDPV isolates to the sequencing lab within three days of obtaining the ITD results. The proportion of the annual results reported by the NRLP during the study period (2010–2018) is shown in Figure 1. All results of AFP cases were communicated by the NRLP within the deadlines set by the WHO. All three standard indicators of laboratory timeliness were met.

4. Discussion

Morocco was certified as polio-free by the WHO in 2015, and no new cases of WPV have been reported. However, the importation of poliovirus is a potential threat as outbreaks of the wild virus occur in the Middle East [11]; therefore, continued surveillance for AFP is essential.

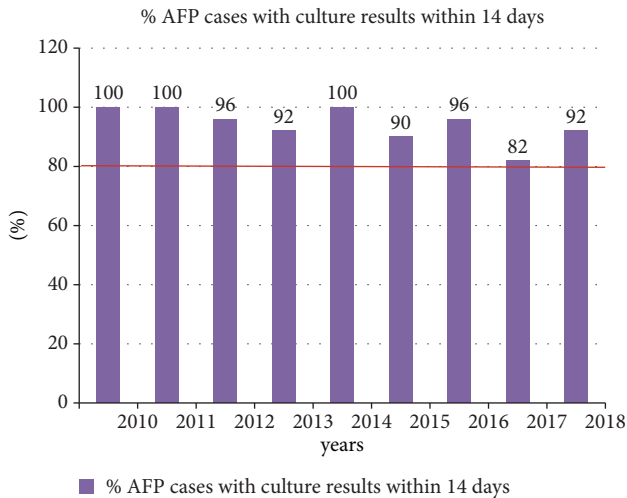


FIGURE 1: Percentage of results of AFP cases reported to the WHO within 14 days of receipt of the sample from 2010 to 2018.

In this study, we reported 11 Sabin-like polioviruses (vaccine strains) after nine years of surveillance of AFP cases and their close contacts as part of the GPEI. The surveillance system revealed no case of WPV or cVDPV in the Moroccan territory. Additionally, 0.6% of cases of residual paralysis were due to attenuated PSL. The data showed that most cases of AFP occurred in children below five years; this was similar to the reports of other studies [12, 13] and confirmed that the risk of developing AFP is greater at a younger age.

The incidence of AFP during the study period was consistently higher among males than females, with an average incidence rate of 56.8% and 43.2% in boys and girls, respectively. Morbidity and mortality rates are higher in men than in women throughout life, and women have stronger humoral and cellular immune responses to infection or antigen stimulation [14]. Our results were similar to those reported in other countries such as Egypt, Jordan, and Spain [12, 15, 16], indicating that boys are more affected by paralysis than girls.

Based on the laboratory data, the reporting of cases of paralysis has improved since 2017 in various regions of the country, especially the Fes-Meknes, Grand Casablanca, Marrakech Safi, and Oriental regions. This improvement is the result of training and awareness workshops conducted by the epidemiologic surveillance service in collaboration with the NRLP. These programs were conducted to ensure that no case of paralysis is missed, regardless of the established diagnosis. Due to these efforts, the AFP detection rate increased, and finally, in 2017 and 2018, met the WHO requirement of ≥ 1 per 100,000 children. However, some southern regions failed to report AFP; hence, more efforts are needed, particularly for polio eradication.

The rate of NPEV isolated in the laboratory is a performance indicator linked to the quality of the specimen and transport conditions. The performance of the quality of viral isolation by cell culture is checked every year through the “proficiency test” for isolation and is always found to be 100%. The NPEV rate was very low and was 2.24% in 2018.

Although the number of cases reported has doubled, the transport conditions have not improved. Similarly, the mean NPEV isolation rate reported was 6% in Jordan [15] and 5% in Kurdistan [17]. A high NPEV isolation rate of 20% was reported in Ghana [18], 15% in Egypt [12], and 14.6% in Nigeria [19]. The NPEV rate is a strict criterion for accreditation of the reference laboratories. Hence, it is important to improve the virological culture technique or use more sensitive detection techniques, such as RT-PCR, which do not require the virus to be viable for detection.

4.1. Limitations of the Study. This study had some limitations. We performed a retrospective programmatic evaluation, and therefore, our study lacked the strength of data fullness. While designing the study, we took measures to limit sources of bias by gathering uniform and full information, mainly for the data in the first year, where the information system was paper-based. The data completeness was checked thoroughly. Consecutively, enrolling AFP cases and data collection depended on the responsiveness of the field epidemiology teams. Catch-up was often performed in the third trimester. Data management improved after implementing the information system, and patient records were generally complete.

5. Conclusion

In this study, we found that the AFP virological surveillance system has improved and has not detected WPV, indicating that the “polio-free” status in our country for the last nine years between 2010 and 2018 is valid. However, the indicators of epidemiological surveillance, including the adequacy of stool samples and the notification of AFP cases, are weak at a regional level and require further efforts for more effective surveillance. The cold chain from sample collection to laboratory testing needs to be maintained to ensure the reliability of the stool specimens required for the isolation of NPEVs.

Data Availability

All data reported in the tables were included in the main manuscript.

Disclosure

This study is a report of AFP surveillance in Morocco and was conducted as part of the routine poliovirus surveillance program in the National Referral Polio Laboratory of the National Institute of Hygiene.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

- [1] M. M. Mehndiratta, P. Mehndiratta, and R. Pande, "Polio-myelitis: historical facts, epidemiology, and current challenges in eradication," *The Neurohospitalist*, vol. 4, no. 4, pp. 223–229, 2014.
- [2] World Health Organization, "World Health organization: immunization," 2019, <https://www.who.int/immunization/diseases/poliomyelitis/en/>.
- [3] A. Rachlin, J. C. Patel, C. C. Burns et al., "Progress toward polio eradication-worldwide, january 2020-april 2022," *MMWR. Morbidity and Mortality Weekly Report*, vol. 71, no. 19, pp. 650–655, 2022.
- [4] S. Wassilak and W. Orenstein, "Challenges faced by the global polio eradication initiative," *Expert Review of Vaccines*, vol. 9, no. 5, pp. 447–449, 2010.
- [5] J. Smith, R. Leke, A. Adams, and R. H. Tangermann, "Certification of polio eradication: process and lessons learned," *Bulletin of the World Health Organization*, vol. 82, no. 1, pp. 24–30, 2004.
- [6] K. Fahmy, L. M. Hampton, H. Langar et al., "Introduction of inactivated polio vaccine, withdrawal of type 2 oral polio vaccine, and routine immunization strengthening in the eastern mediterranean region," *Journal of Infectious Diseases*, vol. 216, pp. S86–S93, 2017.
- [7] S. S. Z. Zaidi, H. Asghar, S. Sharif, and M. M. Alam, "Poliovirus laboratory based surveillance: an overview," *Methods in Molecular Biology*, vol. 1387, pp. 11–18, 2016.
- [8] Moroccan Government Website Concerning the Regionalization: Regionalisationavancee, 2018.
- [9] 2019, <https://www.worldometers.info/world-population/morocco-population>.
- [10] World Health Organisation, *Polio Laboratory Manual*, World Health Organisation, Geneva, Switzerland, 4th edition, 2004.
- [11] A. Wilder-Smith, W. Y. Leong, L. F. Lopez et al., "Potential for international spread of wild poliovirus via travelers," *BMC Medicine*, vol. 13, no. 1, p. 133, 2015.
- [12] A. Abdel-Fattah, A. H. El-Gilany, R. El-Masry, and A. Kanddeel, "Acute flaccid paralysis in North East Delta, Egypt: a retrospective analysis of prospectively collected surveillance data," *Journal of Infection and Public Health*, vol. 12, no. 5, pp. 714–719, 2019.
- [13] H. K. Membo, A. Mweene, S. A. Sadeuh-Mba et al., "Acute flaccid paralysis surveillance indicators in the Democratic Republic of Congo during 2008–2014," *The Pan African Medical Journal*, vol. 24, p. 154, 2016.
- [14] M. Muenchhoff and P. J. R. Goulder, "Sex differences in pediatric infectious diseases," *Journal of Infectious Diseases*, vol. 209, pp. S120–S126, 2014.
- [15] F. Zerriouh, Y. Khader, N. Qasem et al., "Evaluation of the acute flaccid paralysis surveillance system in polio-free Jordan, 2012–2016: retrospective secondary analysis," *JMIR Public Health and Surveillance*, vol. 5, no. 3, Article ID e14217, 2019.
- [16] J. Masa-Calles, N. Torner, N. López-Perea et al., "Acute flaccid paralysis (AFP) surveillance: challenges and opportunities from 18 years' experience, Spain, 1998 to 2015," *Euro Surveillance*, vol. 23, no. 47, Article ID 1700423, 2018.
- [17] J. Soltani, N. Esmailnasab, D. Roshani, M. Karimi, and M. J. Amjadi, "Acute flaccid paralysis and its differential diagnosis in in Kurdistan province, Western Iran; an 11-year surveillance," *Iranian Journal of Pediatrics (Persian edition)*, vol. 24, no. 2, pp. 131–139, 2014.
- [18] J. K. Odoom, N. A. A. Ntim, B. Sarkodie et al., "Evaluation of AFP surveillance indicators in polio-free Ghana, 2009–2013," *BMC Public Health*, vol. 14, no. 1, p. 687, 2014.
- [19] O. B. Soji, O. A. Olayinka, H. O. Tekena, M. M. Baba, D. N. Bukbuk, and O. O. Olayinka, "Non-polio enteroviruses implicated in acute flaccid paralysis in northern Nigeria," *Research Journal of Medicine and Medical Sciences*, vol. 2, no. 1, pp. 25–28, 2007.