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Assessment of areas at increased risk for poliovirus circulation in Ecuador

GUSTAVO H. DAYAN[1]*, RODRIGO RODRÍGUEZ[2], JAN VINJÉ[3], NANCY VÁSCONEZ[4], VÍCTOR CÁCERES[1], NICOLE GREGORICUS[3], MARK SOBSEY[3] AND MAURICIO LANDAVERDE[5]

[1] *Centers for Disease Control and Prevention, Atlanta, GA, USA*
[2] *Pan American Health Organization, Quito, Ecuador*
[3] *University of North Carolina, Chapel Hill, NC, USA*
[4] *Ministry of Public Health, Expanded Programme on Immunization, Quito, Ecuador*
[5] *Pan American Health Organization, Washington, DC, USA*

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SUMMARY

To assess areas at risk for poliovirus circulation in Ecuador, we first selected provinces at highest risk based on low immunization coverage with three doses of oral poliovirus vaccine, and a low number of reported cases of acute flaccid paralysis (AFP). Subsequently, we reviewed discharge data for the period 1996–2000 for diagnoses compatible with AFP in the only two national referral hospitals in Quito, and at least two main hospitals in each of the six selected provinces. Environmental samples from one or two cities/towns in each selected province were tested for poliovirus. Of the 14 identified AFP-compatible cases, 8 (57%) had been previously reported and investigated. We visited four out of the six unreported cases; none of those four had sequelae compatible with poliomyelitis. From the 14 environmental samples taken, we identified Sabin viruses in six of the samples; no vaccine-derived polioviruses were isolated. Using this methodology, we found no evidence of undetected poliovirus circulation in Ecuador.

INTRODUCTION

In 1985 the Member States of the Pan American Health Organization (PAHO) resolved to eradicate wild poliovirus from the Region of the Americas. The strategies used to interrupt transmission of wild poliovirus were achieving and maintaining high levels of vaccination coverage in children <5 years of age (through routine and supplemental immunization activities), developing adequate surveillance systems for acute flaccid paralysis (AFP) cases, and controlling outbreaks by responding rapidly to new cases [1]. Following successful implementation of these strategies, the region's last case of poliomyelitis due to wild poliovirus was reported in 1991, in Peru, and the western hemisphere was certified poliomyelitis-free in 1994 [2].

During 2000–2001, 13 laboratory-confirmed poliomyelitis cases caused by a genetically drifted vaccine-derived poliovirus (VDPV) type 1 were identified in the Dominican Republic, and 8 more cases were identified in Haiti [3]. With one exception, all of the cases were unvaccinated or inadequately vaccinated. This outbreak underscored the necessity of maintaining high levels of vaccination coverage and of surveillance, even where poliomyelitis has been

* Author for correspondence: Dr G. H. Dayan, Centers for Disease Control and Prevention, National Immunization Program, 1600 Clifton Rd, Mailstop E-61, Atlanta, GA 30333, USA.
(Email: gdayan@cdc.gov)

eradicated, and brought to the forefront important questions regarding poliomyelitis vaccination strategy once global eradication is achieved [4, 5].

As part of intensified surveillance activities and as a regional response to the outbreak in the Dominican Republic and Haiti, PAHO developed a protocol to assess countries at increased risk for undetected poliovirus circulation [6]. All countries were categorized on the basis of three key parameters: national immunization coverage with three doses of oral poliovirus vaccine (OPV3), AFP surveillance sensitivity, and the enterovirus isolation rate (i.e. the percentage of stool specimens from reported AFP cases in which enterovirus is isolated), a marker of laboratory performance (target $\geqslant$10%). Using this methodology, Ecuador was identified as a country at increased risk.

We developed a field instrument to identify high-risk areas within a country, and then we tested that instrument in Ecuador. Key objectives of our methodology included: ranking provinces and provincial areas by risk for undetected virus transmission, conducting active searches for previously unidentified AFP cases in the areas at increased risk, evaluating those cases, and testing environmental samples from selected areas for the presence of polioviruses.

METHODS

National data

Located on the northwest coast of South America, the country of Ecuador had a population of approximately 12 647 000 in 2000 [7]. The country has three regions: Costa (Coast), Sierra (Mountain), and Oriente (East), located in the western, central and eastern parts of the country respectively. The country is organized into provinces, which in turn are subdivided into cantons (districts). Poliomyelitis surveillance in Ecuador occurs through a mandatory reporting system based on an AFP clinical case definition. Like other countries in the Region of the Americas, Ecuador reports AFP cases through a PAHO-standardized poliomyelitis surveillance database, the Polio Eradication Surveillance System (PESS) [8]. The target rate for AFP surveillance performance is at least one case of non-poliomyelitis AFP per 100 000 population <15 years [9, 10]. Ecuador exceeded that target rate in all of the years from 1996 to the end of 2000, except for 1998, when a rate of 0·94 was reported. Regarding vaccination coverage for the period 1996–2000, reported national OPV3 coverage rates in Ecuador were below the PAHO target of 80% in two of the years – 1997 (77%) and 1999 (70%) [11]. Moreover, the reported national enterovirus isolation rates in Ecuador were below the targeted 10% rate in 1996 (8%) and 1998 (5%).

Ranking of the provinces

To assess risk at the provincial level we evaluated vaccination coverage and AFP surveillance quality for 1996–2000. OPV3 coverage was considered low if found to be <80%. AFP surveillance quality was assessed in terms of the number of reported *vs.* expected AFP cases with $\geqslant$1 stool specimen collected within 14 days of the onset of paralysis during 1996–2000. The expected number of AFP cases was calculated based on two parameters: (1) annual rate of AFP reported cases per 100 000 children aged <15 years: $\geqslant$1, and (2) proportion of AFP cases with two stool specimens collected within 14 days of the onset of paralysis: $\geqslant$80%.

We used the following formula to calculate the expected number of cases:

$$(\sum \text{population} < 15 \text{ years during last 5 years}/100\,000) \times 0{\cdot}8.$$

Provinces were ranked according to OPV3 coverage and AFP surveillance performance (Table 1). The provinces were stratified into four categories:

Category I (lowest risk): provinces with vaccination coverage above 80% in each of the preceding 5 years (i.e. 1996–2000), irrespective of surveillance quality.

Category II: provinces with adequate AFP surveillance indicators, irrespective of OPV3 coverage.

Category III: provinces with adequate vaccination coverage and surveillance during any, but not all, of the preceding 5 years.

Category IV (highest risk): provinces that did not attain adequate vaccination coverage or surveillance during any year.

Active search and correlation with previous reports to PAHO

From the public health perspective, each province in Ecuador is divided into health areas. Each health area may comprise one or more cantons, or it may be part of a large canton. Vaccination coverage in each health area is reported using the administrative method (i.e. number of administered doses divided by the target population).

Table 1. *Classification of provinces at risk for poliovirus circulation, Ecuador 1996–2000*

		OPV3 coverage (%)[a]					No. of AFP cases[b]	
Category	Province	1996	1997	1998	1999	2000	Expected[c]	Reported[d]
I (Lowest risk)	Azuay	**90**	**89**	**91**	**91**	**90**	9	1
	Carchi	**90**	**100**	**87**	**87**	**84**	**2**	**4**
	Galápagos	**100**	**100**	**100**	**100**	**100**	**0·2**	**1**
	Guayas	**100**	**100**	**82**	**82**	**100**	**43**	**61**
	Sucumbíos	**96**	**80**	**89**	**89**	**83**	**2**	**2**
II	Pichincha	**91**	**90**	76	75	**82**	**30**	**79**
	Tungurahua	**88**	**94**	69	69	**82**	**6**	**6**
	Ríos	**88**	**88**	64	64	73	**9**	**10**
	Pastaza	76	75	74	74	**80**	**1**	**2**
	Chimborazo	77	70	67	67	65	**7**	**10**
	Imbabura	77	72	64	64	70	**5**	**6**
III	El Oro	**100**	**100**	76	76	**94**	7	2
	Morona Santiago	**80**	**100**	n.a.	73	**87**	2	0
	Zamora Chinchipe	69	78	**81**	**81**	77	2	0
	Cañar	79	**100**	60	59	75	3	0
	Manabí	71	**84**	51	51	70	18	12
IV (Highest risk)	Cotopaxi	61	68	50	50	55	5	4
	Esmeraldas	63	61	48	48	69	7	3
	Loja	69	78	59	60	65	6	2
	Napo	55	56	50	50	70	3	2
	Bolívar	68	66	55	55	78	3	0

n.a., Data not available.

[a] Coverage rates with three doses of oral poliovirus vaccine (OPV3): **bold** values represent provinces reporting $\geqslant 80\,\%$ OPV coverage for each year.

[b] Number of acute flaccid paralysis (AFP) cases with two stool specimens collected within 14 days of onset of paralysis: **bold** values represent provinces reporting an equal or higher number of AFP cases than expected during 1996–2000.

[c] AFP expected cases calculated based on two parameters: (1) annual rate of at AFP reported cases per 100 000 children aged <15 years: $\geqslant 1$, and (2) proportion of AFP cases with two stool specimens collected within 14 days of the onset of paralysis: $\geqslant 80\,\%$.

[d] AFP cases with two stool specimens collected within 14 days of the onset of paralysis, reported through the Poliomyelitis Eradication Surveillance System (PESS).

There are two national children's referral hospitals, one in the city of Quito and one in the city of Guayaquil, and one general referral hospital in Quito. Each province has one provincial general hospital as well as cantonal hospitals and smaller health-care facilities or centres. In order to select the places to search for AFP cases and potential poliovirus circulation, we conducted three general activities. First, at the national level, we produced a list of all AFP reported cases at PESS in the preceding 5 years (1996–2000), and viral isolation reported through PESS for all AFP reported cases was checked. We selected the two national referral hospitals in Quito to search for diagnoses compatible with AFP (active search). The national children's referral hospital in Guayaquil was not selected, because active search in that hospital is routinely performed. Next, at the provincial level we selected the provincial hospital and at least one cantonal hospital to be visited for active search. We selected particular cantonal hospitals based on three characteristics of their areas of location: (1) a high population density, (2) lowest OPV3 vaccination coverage, and (3) rates lower than 1 case of AFP per 100 000 population per annum, or no reports of any AFP cases during the preceding 5 years. Finally, at the hospital level, we obtained data by reviewing in-patient hospital discharge data for the preceding 3–5 years. The following five AFP diagnostic rubrics were searched: (1) Guillain–Barré syndrome, (2) transverse myelitis, (3) peripheral neuropathy, (4) traumatic neuritis, and (5) paralysis or equivalent diagnoses such as inability to walk. Information abstracted included patient's name, age, sex, address, telephone number if available, diagnosis, and date of hospitalization. Subsequently, we cross-checked identified cases with those previously

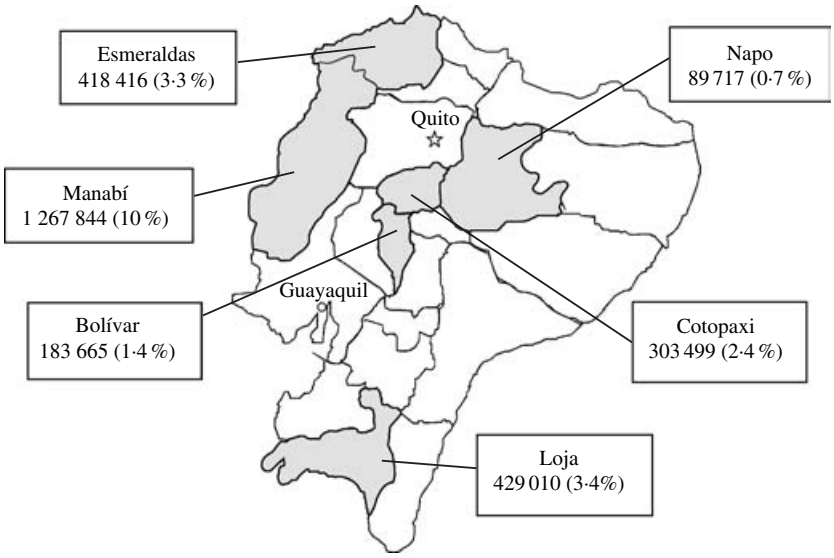

Fig. Ecuador, population in studied provinces and percentage of the total population of the country. □, Study areas.

reported to PAHO through PESS. We also reviewed individual clinical records whenever a previously unreported case was detected. In addition, we attempted to visit all unreported cases in order to assess the presence of poliomyelitis-compatible sequelae.

The methodology was tested in the field during the last week of May and first week of June 2001. Six people divided in two work teams performed all the field activities.

Environmental sampling

In each selected province, we collected 1–3 (1-litre) environmental water samples (e.g. stream water, sewage water) in areas where poliovirus circulation was more likely to be detected. These areas included the ones with the highest population density and the lowest OPV3 vaccination coverage. The number of samples collected depended on the size of the area and the population's access to sewage services. Environmental samples were initially kept in cold boxes with ice packs, then stored at −20 °C, and finally shipped to and analysed at the University of North Carolina (Chapel Hill, NC, USA). The samples were first concentrated by polyethylene glycol precipitation and chloroform extraction and subsequently inoculated into confluent layers of rhabdomyosarcoma (RD) and L20B cell cultures [12], with the latter being more specific for detecting poliovirus.

All cell cultures showing a cytopathological effect (CPE) on L20B cell cultures were tested for poliovirus by microneutralization using pooled polyvalent polio serum [13] and pooled Sabin-specific monoclonal antibodies. A reverse transcription–polymerase chain reaction (RT–PCR) kit from the Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA) was used for intra-typic differentiation. Complete major capsid protein, viral protein 1 (VP1) sequences of all poliovirus isolates were analysed to determine the percentage nucleotide difference compared to the OPV Sabin vaccine strains.

RESULTS

Ranking of the provinces

According to our analysis, the provinces at highest risk were: Bolívar, Cotopaxi, Esmeraldas, Loja, and Napo (Table 1). Although Manabí province did not fall into category IV (highest risk), it was also selected because of its high population, deficient sanitary conditions, poor surveillance, and having only one year with a vaccination coverage rate above 80 %. All three areas of the country were represented (Fig.), with Cotopaxi, Bolívar and Loja in the Sierra area; Manabí and Esmeraldas in the Costa area; and Napo in the Oriente area. The population of the six selected provinces accounted for 21·2 % of the total population of the country.

Active search and correlation with PESS data

The catchment population of the selected hospitals comprised at least 40 % of the total population in

Table 2. *Hospitals selected for active search, areas at increased risk for poliovirus circulation, Ecuador 2000*

Region	Province	Health area (no., name)	Health area catchment population	% of province population[a]	OPV3 coverage 2000 (%)[b]	Type of hospital
National[c]	—	—	—	—	—	National paediatric referral, Quito
	—	—	—	—	—	National general referral, Quito
Sierra	Cotopaxi	1. Latacunga	85 985	28	56	Provincial
		3. Salcedo	49 607	16	36	Cantonal
		2. Pujili	38 937	13	32	Cantonal
		7. Zumbahua	22 380	7	6	Cantonal
		Subtotal	196 909	64		
	Bolívar	1. Guaranda	69 813	38	79	Provincial
		3. Chillanes	22 070	12	33	Cantonal
		Subtotal	91 883	50		
	Loja	1. Centro de Salud 1	53 076	12	43	Provincial
		2. Centro de Salud 2	44 295	10	39	Provincial
		3. Centro de Salud 3	50 720	12	34	Provincial
		7. Macará	26 124	6	41	Cantonal
		Subtotal	174 215	40		
Costa	Manabí	1. Portoviejo	245 734	19	81	Provincial
		2. Manta	219 990	17	99	Cantonal
		3. Chone	157 647	12	49	Cantonal
		Subtotal	623 371	48		
	Esmeraldas	1. Esmeraldas Centro	92 921	22	27	Provincial
		2. Esmeraldas Urbano	91 390	22	40	Provincial
		5. Quinindé	93 849	22	35	Cantonal
		Subtotal	278 160	66		
Oriente	Napo	1. Tena	75 963	85	24	Provincial
		2. Baeza	13 754	15	40	Cantonal
		Subtotal	89 717	100		

[a] Percentage of the province population comprised in the health area (i.e. health area catchment population/province population $\times 100$).
[b] Coverage rates with three doses of oral poliovirus vaccine (OPV3).
[c] National referral hospitals receiving children from all regions of the country.
Source: Instituto Nacional de Estadísticas y Censos [National Institute of Statistics and Census], Ministerio de Salud Pública [Ministry of Public Health].

each one of the six provinces (Table 2). At the two national referral hospitals in Quito, we only searched for patients from the provinces under study. A total of 326 752 diagnoses of hospitalized patients were reviewed. Active search was performed for the period 1996–2000, except in the two cantonal hospitals in Manabí province, where in-patient data were only available since 1998. Of the 14 patients with AFP-related differential diagnoses identified through active search, 8 (57%) had been reported to PESS (Table 3). The six unreported cases were from the provinces of Cotopaxi, Loja, Manabí and Napo. Only two patients could not be visited. None of the four visited cases had poliomyelitis-compatible sequelae (Table 3). Two of the unreported cases occurred in 1996, three in 1998, and one in 2000. The most common diagnosis was Guillain–Barré syndrome, in 4 of the 6 patients. No clusters of unreported AFP cases were detected. Even after inclusion of the newly identified AFP cases, only two provinces (Cotopaxi and Napo) reached the expected number of cases, based on an annual rate of at least one AFP reported case per 100 000 children aged <15 years, for the study period of 1996–2000, and some cases previously reported were not found (Tables 3, 4).

Review of the PESS data regarding previously reported cases showed only one vaccine-associated poliovirus type 1 was isolated from an AFP case reported in Loja in 2000. Three enteroviruses were isolated from reported AFP cases during 1996–2000: one

Table 3. *Cases of acute flaccid paralysis (AFP) found through active search in selected health-care units, Ecuador*[a] *1996–2000*

Province	Hospital	AFP cases found[b]		Non-reported AFP cases visited	Visited cases with polio-compatible sequelae
		Reported PESS[c]	Not reported PESS[d]		
—	National Pediatric Quito[e]	4	0	0	0
—	National General Quito[e]	1	0	0	0
Cotopaxi	Provincial hospital	0	3	3	0
Loja	Provincial hospital	0	1	0	0
Manabí	Cantonal hospital Manta[f]	2	1	1	0
Napo	Provincial hospital	1	1	0	0
Total		8	6	4	0

[a] Hospitals with at least one positive result. All other hospitals (Table 2) were negative.
[b] Total number of AFP cases found through active search in selected health-care units.
[c] Total number of AFP cases found through active search which had been previously reported through the Polio Eradication Surveillance System (PESS).
[d] Total number of AFP cases found through active search which had not been previously reported through PESS.
[e] Only patients from the selected provinces were searched.
[f] Active search was performed for the period 1998–2000.

Table 4. *Number of acute flaccid paralysis (AFP) cases reported through the Polio Eradication Surveillance System (PESS), found through active search in selected health-care units, and total expected cases, Ecuador 1996–2000*

Province	Reported cases (PESS)[a]	Active search[b]	Total detected cases	Total expected cases[c]
Cotopaxi	5	3	8	6
Bolívar	0	0	0	3
Loja	2	1	3	8
Manabí[d]	15	1	16	22
Esmeraldas	4	0	4	9
Napo	2	1	3	3
Total	28	6	34	49

[a] Total number of AFP cases reported through PESS.
[b] Total number of AFP cases found through active search which had not been previously reported through PESS.
[c] Total number of AFP expected cases calculated based on an annual rate of at AFP reported cases per 100 000 children aged <15 years: $\geqslant 1$.
[d] Active search was performed only from 1998 until 2000 in the two cantonal hospitals of Manabí.

in the province of Manabí with a diagnosis of meningitis in 1997, one in the province of Manabí with a diagnosis of encephalitis in 1998, and one in the province of Esmeraldas with a diagnosis of meningitis in 1997.

Environmental sampling

Of the 14 environmental samples collected and analysed for the presence of poliovirus (Table 5), 13 (93%) were CPE-positive on RD, and six (43%) were CPE-positive on L20B cells. Polioviruses were confirmed in all six L20B-positive cell cultures by both neutralization with a pool of type 1, 2 and 3 polyvalent antisera and by RT–PCR using poliovirus-specific primers (Table 5). Sequencing of the complete VP1 genes of these poliovirus strains revealed that they were all identical to the OPV Sabin strains (>99% sequence similarity).

DISCUSSION

We did not find evidence for circulation of wild or VDPV in Ecuador after analysing AFP surveillance data, nor did we isolate wild or VDPV in the environmental samples. However, we were able to test our field instrument within a fairly short time and to identify some deficiencies in vaccination coverage and AFP surveillance.

The sensitivity of our methodology for assessing poliovirus circulation was limited. Indeed, we did not find all reported cases in the hospitals we visited, and not all provinces reached the expected number of AFP cases, even after active search. This may have occurred because some patients may have been seen in

Table 5. *Results of environmental sampling done at selected locations in the selected provinces, Ecuador 2001*

Province	Place, city/town	Source	Collection Date (2001)	CPE on RD[a]	CPE on L20B[b]	RT–PCR results[c]	Phylogenic analysis of VP1 genes[d]
Cotopaxi	Poultier, Latacunga	Sewage	28 May	Pos.	Neg.	Enterovirus	n.a.
	La Cocha, Latacunga	Sewage	28 May	Pos.	Neg.	Enterovirus	n.a.
	La Estación, Latacunga	Sewage	29 May	Pos.	Pos.	Sabin2	Sabin2
Bolívar	Ministerio Agricultura, Guaranda	Sewage	29 May	Pos.	Neg.	Enterovirus	n.a.
	Puente B, Guaranda	Stream water	30 May	Pos.	Pos.	Sabin1, Sabin2, Sabin3	Sabin1, Sabin3
	Puente A, Guaranda	Stream water	30 May	Pos.	Neg.	Enterovirus	n.a.
Manabí	Planta de oxidación, Portoviejo	Sewage	30 May	Pos.	Pos.	Sabin1, Sabin2, Sabin3	Sabin2
	El Salto, Portoviejo	Sewage	30 May	Neg.	Neg.	Neg	n.a.
	Los Gavilanes, Manta	Sewage	30 May	Pos.	Pos.	Sabin2, Sabin3	Sabin2
	Miraflores, Manta	Sewage	30 May	Pos.	Pos.	Sabin1, Sabin2, Sabin3	Sabin1
Esmeraldas	La Barraca, Esmeraldas	Sewage	31 May	Pos.	Neg.	Enterovirus	n.a.
	Isla Piedad, Esmeraldes	Stream water	31 May	Pos.	Neg.	Enterovirus	n.a.
Loja	Virgen Pamba, Loja	Stream water	5 June	Pos.	Pos.	Sabin2, Sabin3	Sabin2
Napo	Pauyshiyacu, Napo	Stream water	7 June	Pos.	Neg.	Enterovirus	n.a.

[a] Cytopathological effect (CPE) on rhabdomyosarcoma (RD) cells.
[b] Cytopathological effect (CPE) on L20B cells.
[C] Reverse transcription–polymerase chain reaction (RT–PCR).
[d] Phylogenic analysis of the major poliovirus capside protein, Viral Protein 1 (VP1).
Pos., Positive; Neg., negative; n.a., not applicable.

other hospitals (i.e. ones not chosen for our active search), some of the patients may have been registered in the outpatient statistics, or the discharge diagnosis may not have been indicative of AFP. Moreover, we were not able to review the in-patient data for the complete period 1996–2000 in two hospitals. However, no wild poliovirus or VDPV was isolated from any of the environmental samples, which makes circulation of poliovirus unlikely. Additionally, we found vaccine viruses in the environmental samples, documenting our ability to pick up polioviruses through our sampling technique.

Maintaining vaccination coverage and surveillance are the major components to prevent and detect re-emergence of poliovirus circulation even after polio-free certification in regions. Population subgroups with low vaccination coverage can sustain the circulation of wild polioviruses for several years within a country [14–16]. Suboptimal immunity contributed to outbreaks of paralytic poliomyelitis related to imported wild poliovirus in The Netherlands in 1978 [17] and 1992–1993 [18], in Canada in 1978 [19], and in the United States in 1979 [20]. More recently, poliomyelitis cases due to importation of wild poliovirus were reported in Cape Verde in 2000 [21] and in Bulgaria in 2001 [22]. Risk factors for circulating VDPVs seem to be similar to those for wild virus [23] as observed in some outbreaks caused by VDPV [24, 25]. However, the threshold rates of vaccine coverage required to prevent circulation of VDPVs remain unknown and may depend on poliovirus serotype and on such environmental factors as population density, levels of sanitation, and climate. While the vaccination coverage rates reported in some areas of Ecuador could sustain the circulation of poliovirus, we found no evidence of such circulation.

Although some countries may have adequate surveillance sensitivity, there may be smaller areas, such as states, provinces, or districts that remain areas of concern. A recent evaluation of the performance of the national poliomyelitis eradication programme in Argentina during 1999–2000 showed regional variations in the sensitivity of the reporting system [26]. This seems to be the case in Ecuador, with some

provinces and areas within the provinces where surveillance could be improved substantially.

Testing of sewage as well as collection and processing of stools from AFP cases and stool surveys of healthy children have also been recommended for surveillance of wild poliovirus circulation [27]. With sewage sampling, there has been good correlation between isolation rates from environmental samples and the number of paralytic poliomyelitis cases occurring in the community [28]. A study carried out in the city of Cartagena, Colombia, revealed that sewage sampling yielded a distribution of virus strains comparable to that obtained with the stool surveys [29]. Our sampling technique has been shown to be useful in detecting vaccine poliovirus despite adverse conditions such as ambient temperatures and high bacterial content of wastewater in tropical areas, which can promote rapid inactivation of polioviruses [30]. Environmental sampling is not currently recommended for surveillance in poliomyelitis-endemic countries. However, targeted environmental sampling may become more important during the final stages of eradication [5].

Our methodology was easy to implement. It identified areas where vaccination coverage and AFP surveillance needed to be improved. As a result of our intervention, supplemental immunization activities as well as training on surveillance and active search were conducted in areas at increased risk. This methodology can be adapted for use in other countries or regions to assess areas at increased risk for poliovirus circulation.

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REFERENCES

1. De Quadros CA, Andrus JK, Olivé JM, et al. Eradication of poliomyelitis: progress in the Americas. Pediatr Infect Dis J 1991; **10**: 222–229.
2. Pan American Health Organization. Final report of the 3rd meeting of the International Commission for the Certification of Poliomyelitis in the Americas. Washington, DC: PAHO, 1994.
3. Kew O, Morris-Glasgow V, Landaverde M, et al. Outbreak of poliomyelitis in Hispaniola associated with circulating type 1 vaccine-derived poliovirus. Science 2002; **296**: 356–359.
4. Sutter RW, Tangermann RH, Aylward RB, Cochi SL. Poliomyelitis eradication: progress, challenges for the end game, and preparation for the post-eradication era. Infect Dis Clin North Am 2001; **15**: 41–64.
5. World Health Organization. Global eradication of poliomyelitis. Report of the sixth meeting of the Global Technical Consultative Group for Poliomyelitis Eradication, Geneva, 7–10 May 2001. Geneva, Switzerland: WHO, 2001.
6. Analysis of country performance of AFP surveillance and vaccination coverage. EPI Newsletter 2001; **XXIII**: 6.
7. Instituto Nacional de Estadísticas y Censos. Población estimada en base a proyecciones de 1990 a 2000 en el Ecuador [National Institute of Statistics and Census. Population estimates based on projections from 1999–2000 in Ecuador]. Quito, Ecuador: Instituto Nacional de Estadísticas y Censos, 2000.
8. De Quadros CA, Bradley SH, Olivé JM, Andrus JK, da Silveira CM, Carrasco PA. Eradication of wild poliovirus from the Americas: acute flaccid paralysis surveillance, 1988–1995. J Infect Dis 1997; **175** (Suppl 1): S37–S42.
9. Pan American Health Organization. Polio eradication field guide. Washington, DC: PAHO, 1994 (Technical Paper No. 40).
10. Strategies for the certification of the eradication of wild poliovirus transmission in the Americas. Bull Pan Am Health Org 1993; **27**: 287–296.
11. Pan American Health Organization. Polio eradication field guide. Washington, DC: PAHO, 1987 (Technical Paper No. 6).
12. Wood DJ, Hull B. L20B cells simplify culture of polioviruses from clinical samples. J Med Virol 1999; **58**: 188–192.
13. Kapsenberg JG. Picornaviridae: the enteroviruses (polioviruses, Coxsackieviruses, echoviruses). In: Barlows A, Hausler, Lenette EH, eds. Laboratory diagnosis of infectious diseases: principles and practice, vol. 2. New York: Springer Verlag, 1989: 692–722.
14. Bernal A, Garcia-Saiz A, Liacer A, de Ory F, Tello O, Najera R. Poliomyelitis in Spain, 1982–1984: virologic and epidemiologic studies. Am J Epidemiol 1987; **126**: 69–76.
15. Conyn-van Spaendonck MA, Oostvogel PM, van Loon AM, Wijingaarden JK, Kromhont D. Circulation of poliovirus during the poliomyelitis outbreak in the Netherlands in 1992–1993. Am J Epidemiol 1996; **143**: 929–935.
16. Strebel PM, Aubert-Combiescu A, Ion-Nedelscu N, et al. Paralytic poliomyelitis in Romania 1984–1992: evidence for a high risk of vaccine-associated disease and reintroduction of wild-virus infection. Am J Epidemiol 1994; **140**: 1111–1124.
17. Bijkerk H. Poliomyelitis epidemic in the Netherlands, 1978. Dev Biol Stand 1979; **43**: 195–206.

18. Oostvogel PM, van Wijngaarden JK, van der Avoort HGAM, et al. Poliomyelitis outbreak in an unvaccinated community in the Netherlands, 1992–93. Lancet 1994; **344**: 665–670.
19. Furesz J, Armstrong RE, Contreras G. Viral and epidemiological links between poliomyelitis outbreaks in unprotected communities in Canada and the Netherlands [Letter]. Lancet 1978; **2**: 1248.
20. Schonberger LB, Kaplan J, Kim-Farley R, Moore M, Eddins DL, Hatch M. Control of paralytic poliomyelitis in the United States. Rev Infect Dis 1984; **6** (Suppl 2): S424–S426.
21. World Health Organization. Outbreak of poliomyelitis, Cape Verde. Wkly Epidemiol Rec 2000; **75**: 401.
22. Centers for Disease Control and Prevention. Imported wild poliovirus causing poliomyelitis – Bulgaria, 2001. MMWR Morb Mortal Wkly Rep 2001; **50**: 1033–1035.
23. Wood DJ, Sutter RW, Dowdle WR. Stopping poliovirus vaccination after eradication: issues and challenges. Bull WHO 2000; **78**: 347–357.
24. Circulation of a type 2 vaccine-derived poliovirus – Egypt, 1982–1983. MMWR Morb Mortal Wkly Rep 2001; **50**: 41–42, 51.
25. Public Health Dispatch: Poliomyelitis – Madagascar, 2002. MMWR Morb Mortal Wkly Rep 2002; **51**: 622.
26. Wolf C, Hidalgo S, Dietz V. Analysis of the performance of Argentina's National Poliomyelitis Eradication Program during 1999–2000. EPI Newsletter 2001; **XXIII**: 3.
27. Pan American Health Organization. Final report of the Second Informal Consultation on Environmental Sampling and Testing Procedures for Wild Poliovirus in the Americas. Washington, DC: PAHO, 1991 (Document EPI/TAG10/92-05).
28. Horstmann DS, Edmons J, Gimpel L, Subrahmanyan T, Riordan JT. Enterovirus surveillance following a community-wide oral poliovirus vaccination program: a seven-year study. Am J Epidemiol 1973; **97**: 173–186.
29. Tambini G, Andrus JK, Marquez E, et al. Direct detection of wild poliovirus circulation by stool surveys of healthy children and analysis of community wastewater. J Infect Dis 1993; **168**: 1510–1514.
30. Hurst CJ, Benton WH, McClellan KA. Thermal and water source effects upon the stability of enteroviruses in surface freshwaters. Can J Microbiol 1989; **35**: 474–480.