



Consolidation of the Neonatal Screening Program as a Public Health Program in Paraguay

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Abstract

In Paraguay, neonatal screening for congenital hypothyroidism (CH) and phenylketonuria (PKU) started in October 1999, in 2005 cystic fibrosis (CF) was selectively incorporated. The National Program for Neonatal Screening has a centralized laboratory that encompasses 1.132 Sample Collecting Sites (SCS) distributed in the 18 Health Regions, with over 80% coverage of live births; the incidence of CH being 1:2.060, HPA/PKU 1:6.328 and CF 1:5.671 newborns. The newborn screening program headed by the Ministry of Public Health and Social Welfare in Paraguay has been consolidated itself as a public health program. This publication describes the historic 20-year process, the strategies and activities carried out as well as the results and achievements, among which it is important to point out the achievement of newborns screening laws that make mandatory to detect, diagnose and treat those affected, as well as the human resources committed to newborn screening.

Keywords: Paraguay, program, neonatal investigation.

Introduction

Newborn screening (NBS) consists of early detection of certain hereditary congenital conditions that may cause cerebral and neurological alterations, growth disorders, digestive and respiratory issues, as well as other severe complications in the development of infants or even death. This test must be performed during the first days of life[1]. In 1962 World Health Organization showed that 3% of the population has a variable degree of intellectual disability, a percentage which may be prevented through newborn screening programs. At first newborn screening was directed towards only one disease, but nowadays over 50 conditions may be detected[2,3]. The diseases investigated during the neonatal period do not present any signs or symptoms and if they do, it only occurs in a low percentage of newborns. That is why neither parents nor pediatricians perceive the health problem, until the damage has been established.

Hence, for more than five decades, newborn screening has become part of the basic care provided in public health in terms of prevention[1]. The newborn screening programs are the result of a collaboration process that involves health professionals, governmental authorities, parents of the affected infants and

the organizations that join them together. These programs must meet requirements that include pre-analytical, analytical and post-analytical stages, which must be well coordinated with each other; systems that include education of parents, health providers and authorities; screening, including sample collection, transport and laboratory analysis; short-term follow-up, tracing and additional analysis when necessary; diagnosis by a specialist physician; patient management, including treatment and medical care; evaluation of long-term follow-up of patients and review of screening systems[4–7].

Pathologies are included in newborn screening programs if they fulfill specific criteria such as high incidence in population, availability of efficient, sensitive, specific, and simple testing, with

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a low number of false positives and if possible, no false negatives, as well available effective treatment, and improved prognosis when treatment starts early[8,9]. According to the population covered programs may be centralized or decentralized.

Paraguay has a land area of approximately 406.750 km², it's a landlocked country, with a population of 7.052.983 and a birth rate of 20,5 per 1.000[10]. It has two official languages, Spanish and Guarani[11]. The country is divided in 17 departments and subdivided in 258 districts. Three of the country's departments are in the Western Region or Chaco with a population of 205.207, and fourteen in the Eastern Region with a population of 6.847.77[12]. The Ministry of Public Health and Social Welfare (MSPBS, by its initials in Spanish) is the governing authority on health, this institution divides the country in 18 Health Regions, made up of the 17 departments, plus Asunción, the capital city, that is the 18th Health Region (Figure 1). The Health Regions oversee the regional and district hospitals, the health centers and health posts and, as of 2009, the Family Health Units (USF, by its initial in Spanish)[13,14].

According to data regarding the number of live births registered in the health indicators, the highest number of births occur in the public health system. In 2018, this percentage was 64,6%; 19,0% at the Social Security Institute Hospitals (IPS- by its initials in Spanish-, the workers' health insurance); 9,7% in private institutions; 5% in other institutions and 1,8% at home [15].

In Paraguay, NBS began in October of 1999, as a research pilot project of the Genetics Department in the Health Sciences Research Institute, called the Program for the Prevention of Mental Retardation (PPRM by its initials in Spanish)[16]. Newborns' dried blood spots (DBS) collected on filter paper test were initiated to establish congenital hypothyroidism (CH) and later, phenylketonuria (PKU) incidence. Both conditions met the inclusion criteria of Wilson and Jungner for population screening[9,16].

The PPRM was recognized by Law 2138 in 2003[17], incorporating cystic fibrosis (CF), to the two former conditions, which resulted in the "Cystic Fibrosis and Mental Retardation Prevention Program" (PPFQRM, by its initials in Spanish).

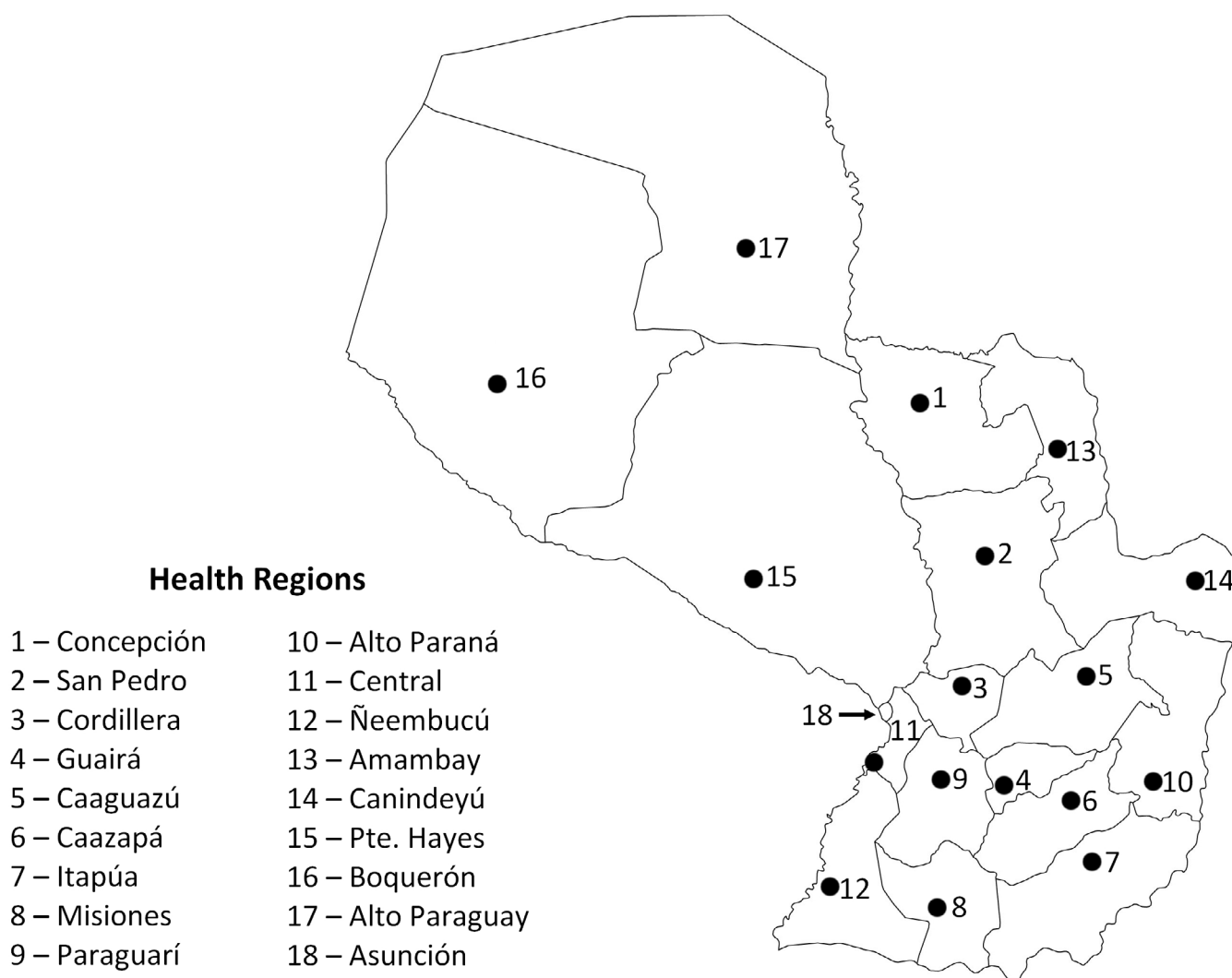


Figure 1. Map of Paraguay showing the eighteen Health Regions.

In 2004, the PPFQRM was incorporated into the MSPBS by a presidential decree[18] and in January 2005 the Senate granted it an independent Budget.

This publication describes the historic 20-year growth of NBS in Paraguay. It includes the strategies and activities carried out as well as the results and achievements obtained.

Methodology

It is a retrospective, descriptive, cross-sectional analysis that reviews the main activities, strategies, achievements and results obtained to implement and consolidate the newborn screening in Paraguay.

Sample Collection Site (SCS): It is a site enabled in a health facility (Regional or District Hospital, Health Centers and USF), where health professionals were trained in collecting the blood sample for NBS and sending it to the PPFQRM for analysis. It also helps finding newborns with positive screening results, as well as spreading information about NBS to the expectant parents, during the prenatal visits.

Sample collection: Samples were collected at the SCS. DBS obtained by heel puncturing from newborns at 24 hours after birth, ideally until 1st week of age for the first sample, until 2 to 4 weeks of age for a second sample (low birth weight, preterm infants, twins, and critically ill infants). Blood spots were dried at room temperature (between 18 and 25 °C) for 2 to 4 hours, and then sent for analysis. The age of the newborn at the time the sample was taken was informed, as well as the number of samples that did not get a result because the sample did not elute, and the samples rejected for the five-year period 2015-2019.

Codification and test: samples were encoded and tested, usually within 24 to 48 hours after arrival to the PPFQRM. All procedures followed the National Guide for Newborn Screening Sample Collection[19].

Coverage estimation: the database of the newborn screened per year was analyzed, comparing the number of registered births, from year 1999 to 2019. In addition, the coverage per Health Region for 2018 was analyzed.

Cut-off values: The cut-off values used in the laboratory for the analysis of the samples at the beginning of the Program were those according to the reagent informed reference value; subsequently these were obtained by statistical analysis for each of the determinations carried out, in our own population[20,21]. Thyroid Stimulating hormone (TSH) was measured for CH and the cut-off value was established at 10 μ UI/ml in DBS. For persistent hyperphenylalaninemia and forms of PKU (HPA/PKU), phenylalanine (Phe) level was measured, whose cut-off values corresponded to 2 mg/dL and for cystic fibrosis (CF), immunoreactive trypsin (IRT), with a cut-off of 55 ng/ml. For TSH and IRT we used AutoDELFIA kits of PerkinElmer®, and for Phe we use an in-house fluorometric assay, based on a modification of the method of Robins and McCaman, from what described by Yamaguchi et al. [1,22]. The laboratory participates in two external quality control programs: “Programa de Evaluación Externa de Calidad” of Federación Bioquímica

Argentina (La Plata, Argentina) and “Newborn Screening Quality Assurance Program” of the Centers for Disease Control and Prevention (Atlanta, USA).

Diagnostic confirmation methods: If an abnormal TSH, Phe or IRT was obtained in DBS it was confirmed with a second sample. In these cases, the family was contacted, and a second sample was requested. We defined an individual as a “case” when its first sample’s test result was higher than the cut-off value and confirmed with a second sample. For CH, the confirmatory tests were the determination of TSH and T_4 in serum; for HPA/PKU confirmatory test was Phe level by fluorometry until 2018, later by MSMS. For CF the confirmatory study was through the sweat test.

Registry of confirmed cases: Once the disorder was confirmed through the respective diagnostic methods, the newborn’s data became part of the Patient Registry. The number of cases detected from October 1999 to October 2020, by Health Region, was analyzed.

Treatment and follow up: the treatment of positive cases is carried out by specialists, according to the pathology. For CH, an endocrinologist is available and laboratory studies of serum TSH and T_4 are offered, and the delivery of sodium levothyroxine and in some cases other drugs, such as zinc sulfate or ferrous sulfate. Patients with HPA/PKU are cared for by a gastroenterologist, the concentration of Phe in the blood is measured, parents are instructed to collect the sample at home and send it for analysis, along with patient food records between visits. Milk without phenylalanine is provided, in the amount recommended by the specialist. Zinc sulfate and ferrous sulfate are also supplied for the correct growth of individuals. In CF cases, as of 2008, pancreatic enzymes, zinc sulfate, ferrous sulfate, azithromycin, salbutamol, tobramycin, and alpha dornase are provided. Nebulization masks and equipment are also supplied as well as equipment to encourage coughing.

Incidence: The incidence was calculated based on the data of the number of newborns diagnosed for the disorder on the number of newborns screened, corresponding to the five-year period 2015-2019, for CH, HPA/PKU and CF. In addition, the age at the beginning of treatment and the predominant gender by pathology were analyzed for the same period.

Results

Since its inception, the NBS in Paraguay was conceived as a centralized program. In 2000, the first working algorithm was available, which defined the organizational structure from the sample collection to the closing of the case (Figure 2).

In 2005, an agreement was signed with the National Postal Service of Paraguay[23], which allowed the free transport of samples from the SCS to the Program and vice versa, the normal results reports and the sample collection forms. The arrival of samples for analysis was as follows: 62% of the samples were sent through the national postal service, 28% by members of the Health Regions where they came from and 10% by private mail.

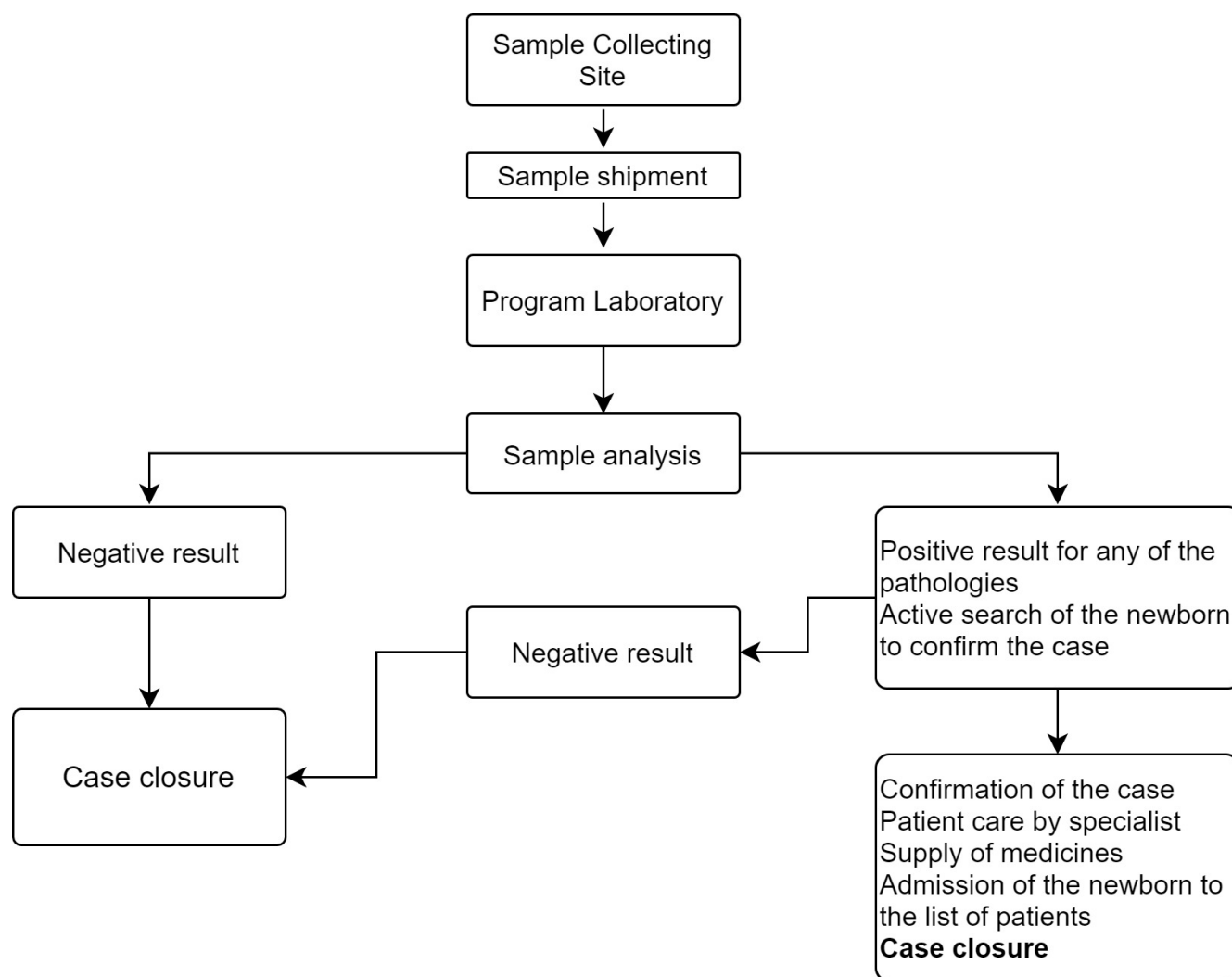


Figure 2. NBS Program Flowchart.

In 2005, supported by Japan International Cooperation Agency (JICA)[24], the project called Establishment of Neonatal Screening Systems in Congenital Hypothyroidism started, aiming program's health and administrative staff training as well as implementation of fluorometric methods for newborn screening.

A second project supported by JICA called "Project for the Reduction of Intellectual Disability in Paraguay", assisted in the preparation of radio and television advertisements in Spanish, Guaraní and sign language, to disseminate the NBS to the general population. The National Guide for the Collection of Newborn Samples [19] and the National Clinical Guide for the care of CF[25] were also developed, as well as brochures on CH, PKU and CF for parents of affected children. JICA also sponsored training in reagents manufacture for determination of Phe, in 2011 after methodology validation, 90% of the reagents were manufactured locally.

Figure 3 shows the sustained increase in coverage, which goes from 0,4% of screened newborns (393/90007) in 1999 to 79,6% (85921/107997) in 2019.

Figure 4 shows the Health Region inclusion by year (continuous line), the bars indicate SCSs number covered by year. By 2012, the eighteen Health Regions had SCSs.

Table 1 shows that 83,3% (15/18) of the Health Regions had a coverage of over 70% and one of them at over 100%, in 2018.

As a result of NBS, from October 1999 to October 2020, 1012863 newborns were screened. Table 2 shows that 81,6% (1132/1388) of the Ministry's health services had installed a SCS, in each district there are a minimum of three SCS. 539 cases of HC were identified, distributed in 100% of the Health Regions, whereas 137 cases of HPA/PKU were distributed in 94,4% (17/18) and 201 cases of CF, distributed in 88,8% (16/18) of the Health Regions.

Figure 5 shows the percentages of samples that did not elute (mean 0,80%) as well as the rejected samples (mean 1,52%) between the years 2015 to 2019. During the 2015-2019 period, 83,0% of newborn screened were between 1 to 5 days old, and 90,0% of the samples were taken before the newborn was 15 days old.

Figures 6, 7 and 8, show the age at treatment onset of diagnosed newborn during the five-year period 2015-2019.

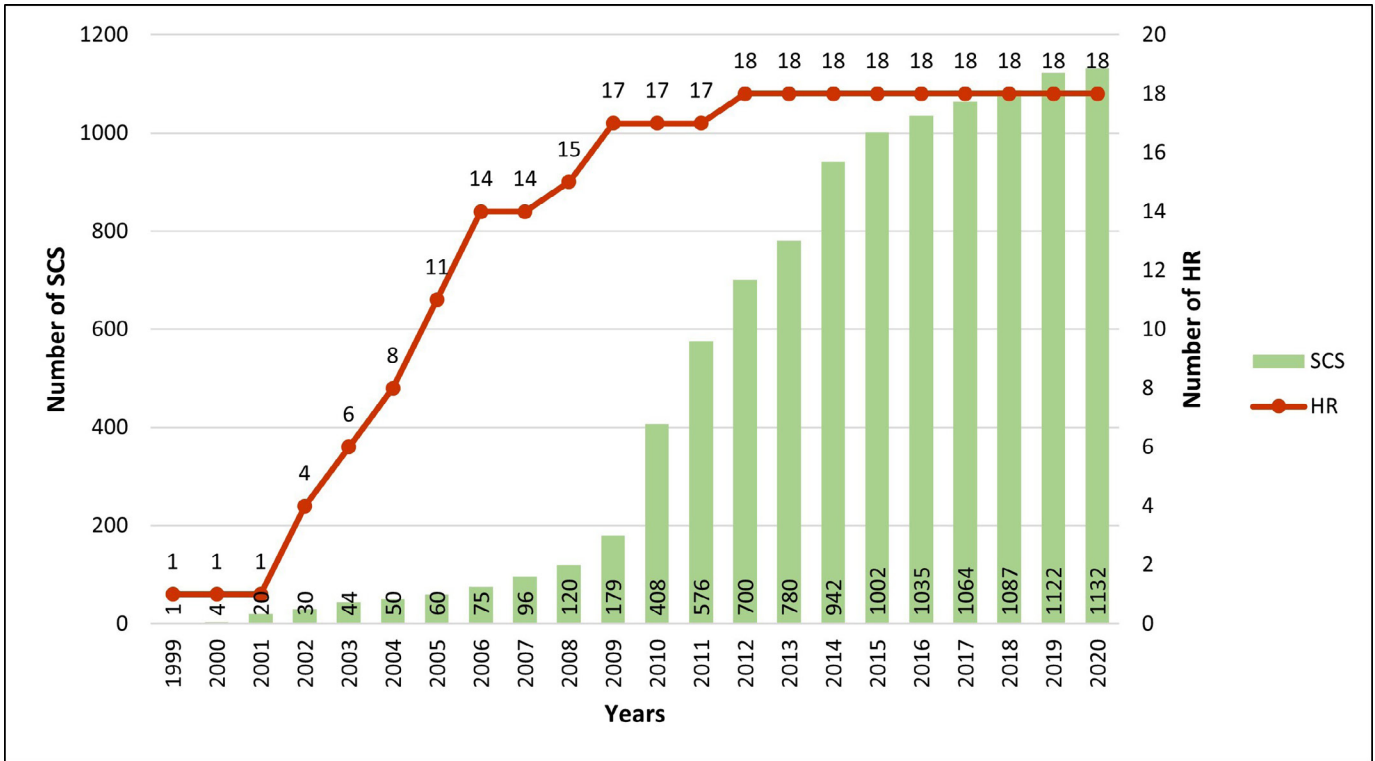


Figure 3. Timeline of SCS and Health Region coverage per year. Abbreviations: SCS: Sample Collecting Site; HR: Health Region.

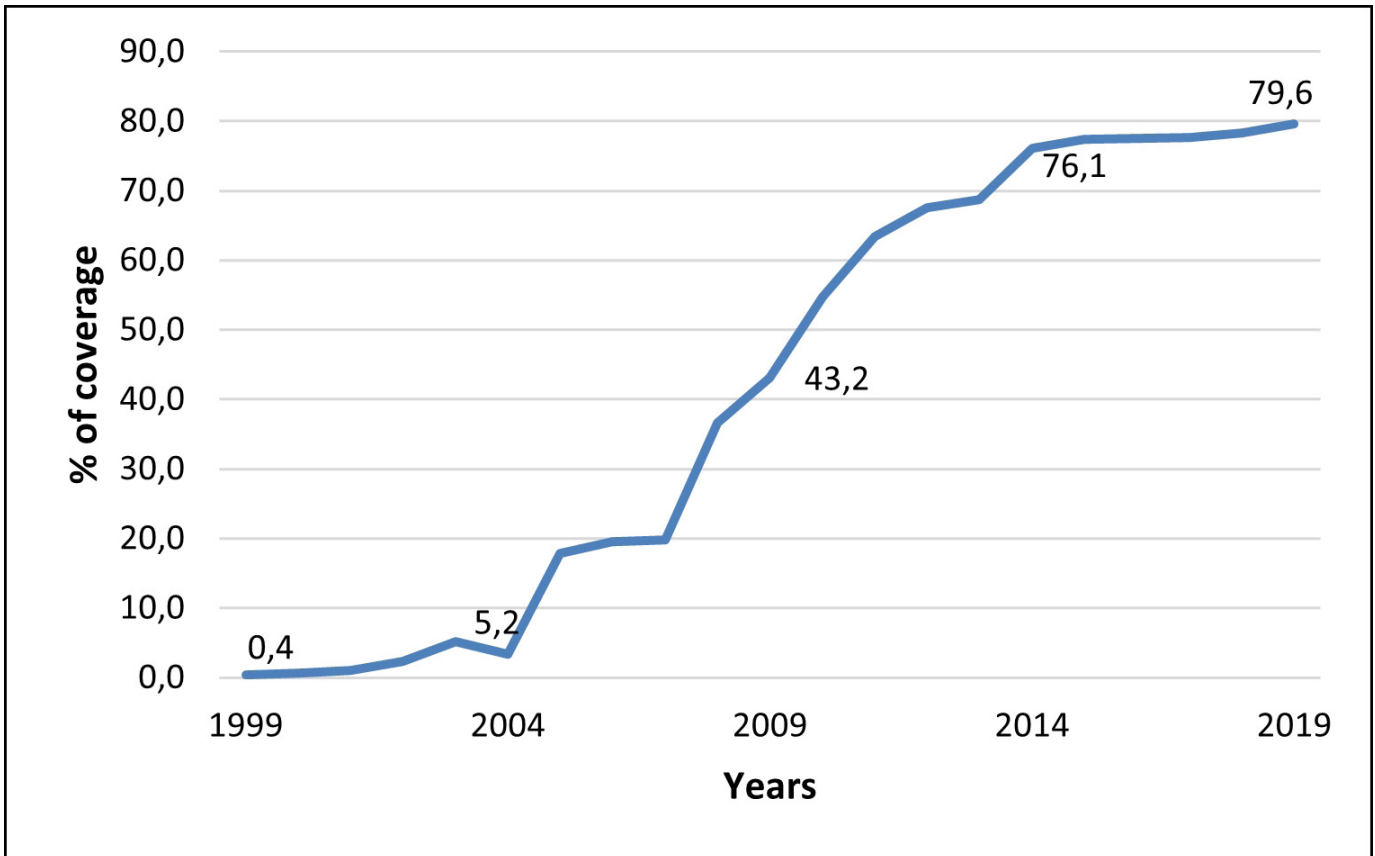


Figure 4. Percentage of coverage, 1999 - 2019.

Table 1. Coverage per Health Region. Year 2018.

Health Regions	Registered newborns *	Screened newborns **	%
1 – Concepción	4399	3262	74,2
2 – San Pedro	6884	4823	70,1
3 – Cordillera	4201	3207	76,3
4 – Guairá	2879	2588	89,9
5 – Caaguazú	8218	6693	81,4
6 – Caazapá	2311	1955	84,6
7 – Itapúa	7525	5842	77,6
8 – Misiones	1871	1730	92,5
9 – Paraguari	2893	2063	71,3
10 – Alto Paraná	15390	12038	78,2
11 – Central	34725	23440	67,5
12 – Ñeembucú	883	818	92,6
13 – Amambay	3106	1658	53,4
14 – Canindeyú	3566	2796	78,4
15 – Pte. Hayes	2199	1161	52,8
16 – Boquerón	1632	1425	87,3
17 – Alto Paraguay	320	226	70,6
18 – Asunción	8640	11694	135,3
Totals	111642	87419	–
		Mean	79,7

* Paraguay's Basic Health Indicators, 99-2019

**Source: National Program for Neonatal Screening Database

Table 2. Data by Health Region on the number of SCS, cases detected and in treatment, by pathology from October 1999 to October 2020.

Health Region	Population *	Number of Districts*	Number of Health Facilities*	Number of SCS **	CH Cases detected and under treatment **	HPA/PKU cases, detected and under treatment **	CF cases detected and under treatment **
1 – Concepción	247675	12	74	71	21	2	4
2 – San Pedro	424774	21	133	86	26	2	6
3 – Cordillera	303242	20	68	71	24	3	6
4 – Guairá	223104	18	84	71	18	2	5
5 – Caaguazú	551774	22	86	81	20	10	12
6 – Caazapá	187035	11	67	56	8	1	7
7 – Itapúa	600011	30	101	93	30	7	16
8 – Misiones	124954	10	68	41	6	3	4
9 – Paraguari	256224	18	74	55	13	3	8
10 – Alto Paraná	808172	22	113	120	49	12	17
11 – Central	2115174	19	155	128	210	41	75
12 – Ñeembucú	89290	16	74	61	10	3	0
13 – Amambay	167050	6	25	23	6	4	3
14 – Canindeyú	226111	16	87	66	16	2	2
15 – Pte. Hayes	123361	9	57	40	4	1	2
16 – Boquerón	64298	3	31	19	2	0	0
17 – Alto Paraguay	17548	4	24	22	8	2	1
18 – Asunción	523184	1	67	28	68	39	33
Totals	7052981	258	1388	1132	539	137	201

* <http://portal.mspbs.gov.py/digies/wp-content/uploads/2020/01/Indicadores-Basicos-de-Salud-IBS-2019.pdf>

**Source: National Program for Neonatal Screening Database

Abbreviations: SCS: Sample collection sites, CH: Congenital hypothyroidism, HPA/PKU: Hyperphenylalaninemia and Phenylketonuria; CF: Cystic Fibrosis.

For CH, 52,4% were diagnosed before 31 days, the average age at the time of diagnosis and treatment onset was 33 ± 20 days and a median of 28. For HPA/PKU, during the same period 33,3% were diagnosed before 31 days, the average age was 48 ± 27 days at the time of diagnosis and start of treatment with a median of 43 days. If we separate in HPA with Phe values between 2 and 6 mg/dL, the average age at the time of diagnosis and start of treatment were to 51 ± 28 days, with a median of 45 days, and for PKU with values of Phe higher than 6 mg/dL, the average age was 30 ± 18 days, the median being 22 days. For CF, only 14,2% were diagnosed before 31 days, the average age at the time of diagnosis and start of treatment was 73 ± 42 days, with a median of 67 days.

Regarding the incidence by pathology for period 2015-2019, the average incidence for CH was 1:2.060 newborns. While the average combined for HPA/PKU is 1:6.328 newborns, of the 69 newborns detected and monitored, 12 corresponded to PKU that required nutritional treatment. The average incidence for CF was 1:5.671 newborns. For the same period, the gender distribution for CF was 42,9% females (33/77) and 57,1% males (44/77); for HPA/PKU was 52,2% females (36/69) and 47,8% males (33/69), in contrast to the distribution for CH, where 28,8% (61/212) corresponded to males, and 71,2% (151/212) to females, a proportionality of 1M:3F.

Other important achievements to be mentioned were, achieving through a Presidential Decree N°864 in 2013 that

the third Sunday in December was set to commemorate the “National Day of fight against CF”[26]. Then in 2016, Law 5732/2016 changed the Program’s name to the “National Program for Newborn Screening” (PNDN, by its initial in Spanish) [27], to extend the screening panel to include: galactosemia, congenital adrenal hyperplasia, biotinidase deficiency, as well as other inborn errors of metabolism. For this reason, in that year, a tandem mass spectrometry equipment was acquired, for aminoacidopathies, fatty acid oxidation defects and organic acidurias screening, used initially for samples from symptomatic individuals, and implemented in the beginning at one hospital.

Another achievement between 2015 to 2017 types of mutations were analyzed of 89 CF patients[28,29].

The last point, Law 6864/2019 created the “National Program for the Comprehensive Care of Individuals with CF”[30], and Center for CF patients care, assistance and follow up was opened at the Acosta Ñu Children’s Hospital.

Discussion

Under the name of PPRM, in 1999 the neonatal screening program was started in Paraguay, as a pilot project in a hospital in the 3rd Health Region, an area known to have a high prevalence of Iodine Deficiency Disorders. The algorithm developed during the first years of the Program is still in use. With Law 2138, neonatal screening, diagnosis, and treatment of newborns

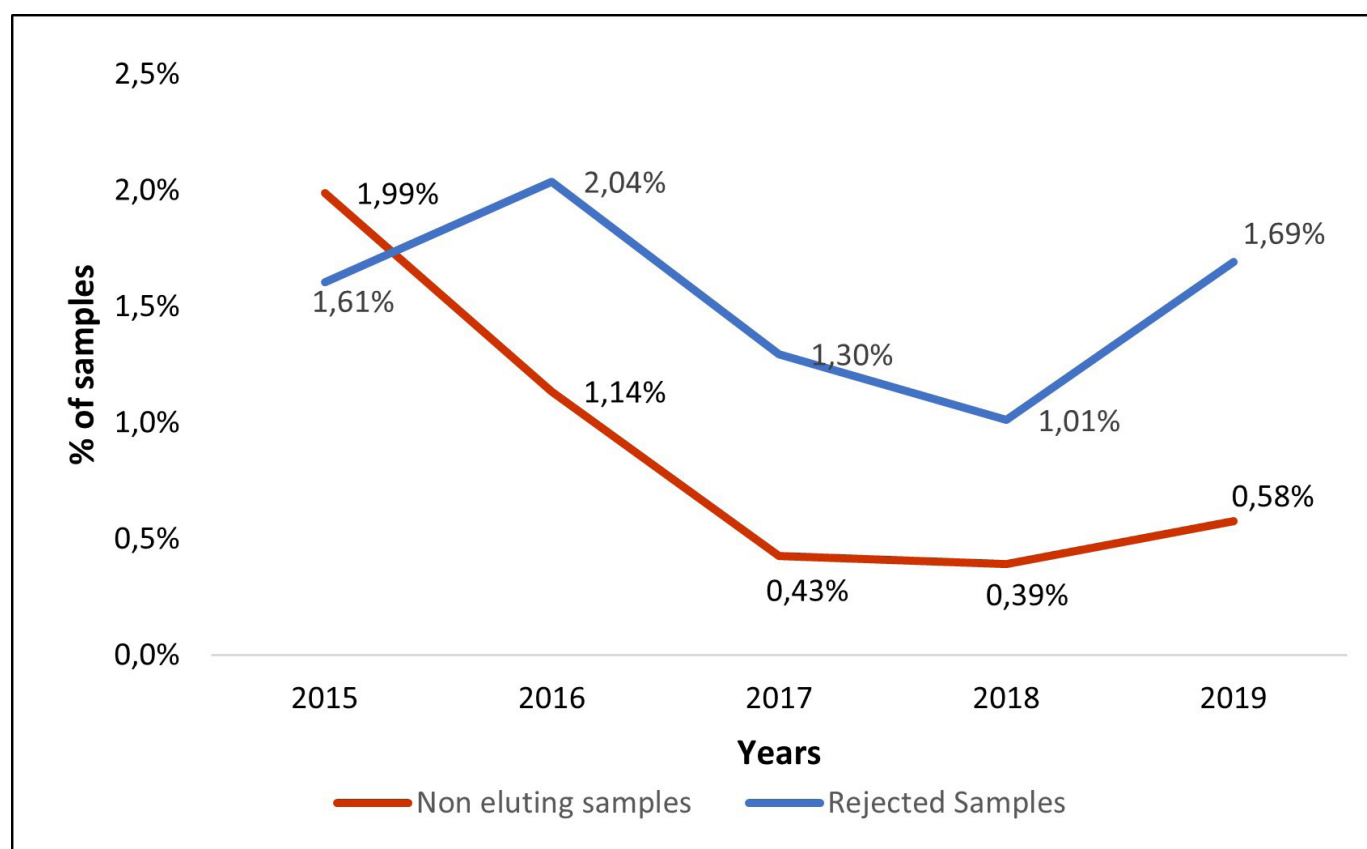


Figure 5. Non eluting and rejected samples, 2015 – 2019.

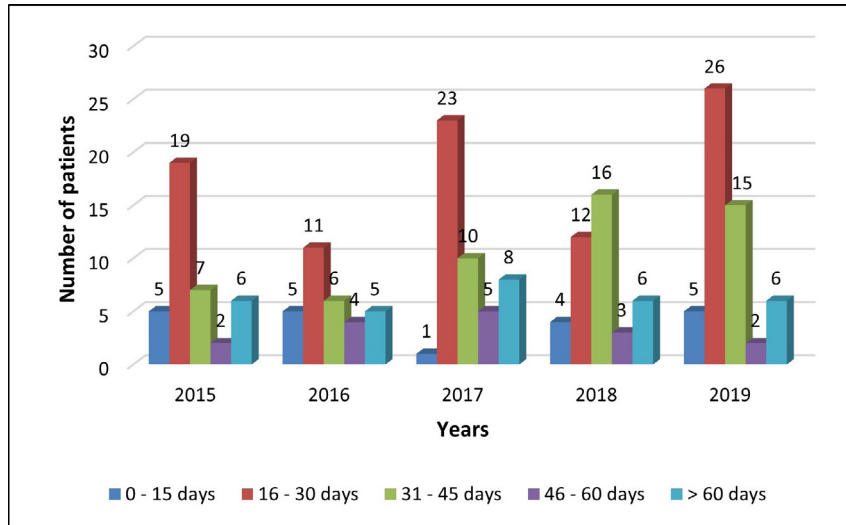


Figure 6. Age of onset of treatment for infants diagnosed with CH.

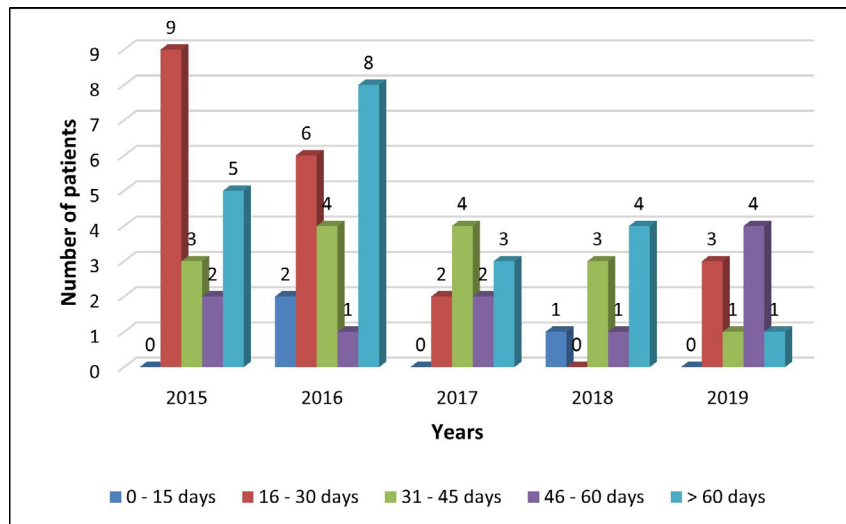


Figure 7. Age of onset of treatment for infants diagnosed with HPA/PKU.

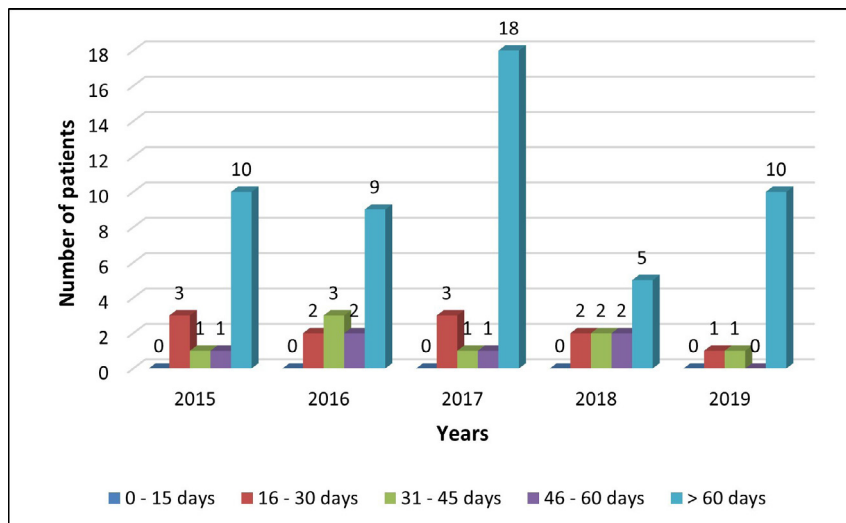


Figure 8. Age of onset of treatment for infants diagnosed with CF.

detected as well as cases diagnosed in previous years became mandatory and free of charge[16,17].

The agreement signed between the MSPBS and the postal service of Paraguay, similar to the one signed by the NBS program in Uruguay, was key to the expansion of the program, as it allowed the new program an active exchange of samples and supplies through the 261 post offices distributed in the eighteen Health Regions[23,31].

The support received from JICA was crucial: firstly, the training of program professionals; secondly, the change of methodology to one of greater sensitivity and specificity; thirdly, the increase in coverage with the inclusion of the missing Health Regions and the opening of new SCSs, reaching 17 of the 18 Health Regions in the country. After the opening of a new SCSs, it is important to communicate to the general population about the program, as mentioned by Torresani and Therrel[4,5], this was achieved through meetings and conferences, promotion through the media and social networks, as well as the distribution of informative brochures[32,33]. The good performance demonstrated during the years of support, added to an evaluation in a group of pregnant women, on knowledge of neonatal screening[34], allowed us to access a last support, this time for the in-house manufacture of a reagent to measure phenylalanine.

It is noteworthy a sustained growth, going from an initial coverage of 0,4% to reach in the last five years percentages higher than the estimated for the population born in the health services covered by the MSPBS, which in 2018 was 64,6%[15], while the number of newborns screened for the same year was 79,7%. It has been possible thanks to the inclusion of both the Health Regions and the SCSs opened in these places, as visualized in Figure 4. We observe a plateau that remained stable with values higher than the number of births occurring in MSPBS, so we assume that these screened newborns correspond to births occurring in dependencies other than those of MSPBS services. A clear example of this can be seen in Table 1, in the 18th Health Region, where coverage reaches a value higher than 100%, which could be due to two reasons, one that these newborns came from pregnant women from other Health Regions, referred to the capital for delivery, or from newborns in private services, who resorted to the program for their neonatal screening.

We believe that the fact that each district has a minimum of 3 SCSs made it possible to detect CH cases in 100% of the health regions, especially considering the high incidence of this pathology in the Paraguayan population. Since one of the main objectives of the Program is to attend the most vulnerable populations, we can see that the total number of populations corresponds to the number of SCS, the larger the population, the larger the number of SCSs, with the capital city being an exception to this rule, since maternity hospitals are more centralized.

Weaknesses include the age of diagnosis and initiation of treatment, mainly associated with the delay in locating positive

cases after screening and in CF the diagnostic sweat test performed once a month. However, an improvement is seen with respect to what was published in the first decade of the program in relation to CH, with the average age at start of treatment being 50 days[35]. We should continue monitoring until treatment is started before two weeks[36], considering the high incidence of this pathology, as well as others such as the rejection of samples either by non-elution or insufficiency, being a strategy the continuous training of health professionals, if possible, included as an undergraduate subject in public health careers.

The incidence of the pathologies detected is similar to what is described in other countries in the region. It is important to point out the proportion of the female over the male gender that is prevalent in CH, being 3:1, this has already been reported[35,37–39].

Conclusions

The work done throughout more than two decades, through training and information dissemination, to health professionals as well as to the general population and the country's authorities, has paid off, allowing us not only to have a presence in the eighteen Health Regions with a network of 1132 SCSs, but also allowed the detection and treatment and follow up of 539 newborns with CH, 137 cases of HPA/PKU and 201 with CF, all of this is endorsed by laws and regulations. We remain committed to include other pathologies and above all to establish a system for assessing the quality and performance of all the entities involved in newborn screening, which will allow us to identify the gaps to be covered, to offer a quality National Neonatal Screening Program.

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Declaration of Conflicting of Interests

The authors declare no conflict of interests.

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