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A newborn screening pilot for sickle cell anemia and congenital hypothyroidism in Guyana

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Abstract

Introduction: Newborn screening (NBS) is a test done shortly after birth to detect conditions that cause severe health problems if not treated early.[1] Approximately 71% of babies worldwide are born in a jurisdiction that does not have an established NBS program. [2] Guyana currently has no NBS program and has established a partnership with Newborn Screening Ontario (NSO) to initiate screening.

Objectives: to assess the feasibility of implementing a NBS program in Guyana for congenital hypothyroidism (CH) and hemoglobinopathies (HBG) and to report on screen positive rates and prevalence (Hardy-Weinberg equilibrium (HWE) for CH and HBG.

Methods: Term, healthy Guyanese infants were tested (with consent) using heel prick dried blood spots (DBS) shortly after birth (closer to 24 hours of life). DBS samples were analysed at NSO. Screening test for CH was done using a human thyroid-stimulating hormone (hTSH) assay. Mean

hTSH levels between the Guyanese sample and the Ontarian population were compared using Student's T test with an alpha of 0.05. Screening test for SCD was performed with a cation-exchange high-performance liquid chromatography (HPLC).

Results: The pilot was conducted from June 6th, 2016 to September 22nd, 2017. GPHC recruited 2,294 mothers/infants. Screen positive rate for CH in our sample was 0.0% (0/2,038 infants). Mean TSH levels in Guyanese samples (1.7 µU/mL blood) was noticed to be significantly different than in the Ontario population (4.3 µU/mL blood) ($p < 0.05$). Screen positive rate for SCD in our sample was 0.3% (7/2,039 patients), and the carrier rate was 8.41% (172/2,039patients). Using the HWE, the SCD frequency is (S allele frequency) ^{2} = 0,49 ^{2} = 0.002.

Conclusion: NBS for CH and SCD in Guyana could be beneficial. Future work should focus on conducting larger pilots which could be used to inform diagnosis and treatment guidelines for Guyanese people.

Article Summary

- What is already known? Newborn screening has been shown to reduce the impact of certain diseases that can be detected at birth and managed appropriately.
- What are the new findings? This study shows that there is a prevalence Sickle Cell Disease and carriers in Guyana at rates similar to the rest of the Caribbean.
- What do the new findings imply? The Guyanese population can benefit from implementation of newborn screening for Sickle cell anaemia.

Strengths and limitations of this study

This is the first study on newborn screening to be conducted in Guyana. It provides baseline data for sickle cell anemia and for thyroid hormone levels for newborn babies in Guyana. Results are comparable to that of other Caribbean territories with similar ethnicity. Data can be used by policy makers to justify future work in newborn screening in Guyana.

Limitations include lack of previous baseline data for comparison and interpretation especially with baseline thyroid hormone levels. Relatively small sample size and single centre study due to limited of resources. Difficulty with follow- up of borderline or positive results due to geographic and constraints

Data sharing statement

Extra data is available and can be accessed by emailing pheonar@yahoo.com.

Introduction

Newborn screening (NBS) is a screening test done shortly after birth to detect conditions that cause severe health problems if not treated early.[1]NBS programs have been existing for over 50 years in developed countries.[2]It allows clinical providers to identify infants at high risk for those conditions, provide diagnostic testing and initiate treatment to avoid severe outcome and to prevent death.[3]Conditions included in NBS need to have a relatively high prevalence and their testing and treatment need to be simple, inexpensive and effective.[2]While the number of conditions included in NBS panels varies across jurisdictions, the most advanced laboratory settings give NBS programs the capability for screening for over 50 conditions.[3]

NBS programs in high income countries have made significant advances thanks to the advent of centralized laboratory services and advanced analytical methods, such as tandem mass spectrometry, which has increase the NBS tests' sensitivity and rapidity.[4]In lower- and middle-income jurisdictions, barriers such as lack of resources and infrastructures have limited the development of NBS programs.[4]Approximately 71% of babies worldwide are born in a jurisdiction that does not have an established NBS program.[2] As such, the majority of infants born worldwide with diseases for which NBS exists do not get diagnosed and treated early. This leads to significant mortality, morbidity and economic burden of NBS-eligible diseases in jurisdictions where NBS is not developed.

Guyana currently has no NBS program and has established a partnership with Newborn Screening Ontario (NSO) to initiate screening. NSO was established in 2006 and coordinates NBS for the province of Ontario, Canada. NSO screens over 140,000 newborns per year for 29 targeted diseases by collecting and analyzing dried bloodspot (DBS) samples in the first days of life. Congenital hypothyroidism (CH) and sickle cell disease (SCD) are two relatively common conditions, that are good candidates for NBS and offered by NSO. In the Guyanese population, the prevalence of CH and SCD are unknown but there is evidence they have a significant public health burden, making these two conditions good candidates for an NBS pilot in Guyana.

CH is an endocrine condition that can result in growth deficiency and severe intellectual disability if left untreated.[1]It is one of the most common preventable cause of intellectual disability with an incidence in newborns between 1:2000 and 1:4000, and as high as 1:600 newborns in iodine deficient areas. [1]CH is most often permanent and caused by an abnormality in thyroid gland development or a defect in thyroid hormonogenesis. [5]In some cases, the condition is transient, in which case it can be due to the transplacental passage of maternal medication, maternal blocking antibodies, iodine deficiency or excess. [5]Treatment for CH is effective, inexpensive and easy to manage for families.[1]Infants diagnosed with CH near birth can start treatment and avoid any growth delay or intellectual disability and develop normally.[1]However, when CH patients are identified later, treatment does not suffice to eliminate developmental and growth delays resulting from CH, which underlines the importance of NBS for CH. [1]CH was added to NBS programs starting in the mid-1970s, and has shown to be effective in reducing morbidity, mortality and the economic burden of CH: the jurisdictions screening for CH have eliminated neuro-developmental impairments resulting from CH.[2]Since establishing NBS for CH, Cuba, Puerto Rico and the US Virgin Island have been screening more than 95% of their population for CH and reported a birth incidence ranging

from 1: 3,600 and 1: 9, 526. [2]In Brazil, the CH prevalence has been reported as 1: 2,259. [2]The CH prevalence in Guyana remains unknown.

In Guyana, SCD was found to be among the top leading causes for admission to pediatric wards. During a one-year study, it accounted for 102 of 1380 admissions.[6]SCD is a group of recessive inherited conditions that affects the formation of haemoglobin and the functioning of red blood cells. SCD patients experience a wide range of adverse outcomes, including, but not limited to: anaemia, stroke, ischemic organ damage, pain crises, chronic respiratory disease, recurrent infections, and death. [7]The gene mutations responsible for SCD are most common in populations of African, South Asian, Middle Eastern and Mediterranean origins.[8]SCD is one of the most common genetic conditions worldwide, with a birth incidence estimated at 24,900 cases per year. [8]SCD is a global health public concern: a survey conducted by the World Health Organization reported that haemoglobin disorders such as SCD are a public health concern for 71% of the 229 countries surveyed, who represent 89% of births worldwide. [8]While the prevalence of SCD in Guyana in unknown, studies have been conducted to determine the SCD trait frequency rate and birth prevalence in other Caribbean jurisdictions using the Hardy-Weinberg equilibrium. It is estimated that the SCD birth prevalence is 2.7% in Aruba and 6.8 % in Hardy-Weinberg equilibrium. It is estimated that the SCD birth prevalence is 2.7% in Aruba and 6.8 % in St. Maarten. The SCD trait frequency rate is estimated to be 2.7% in Aruba, 7.3% in St. Maarten, 7% in Barbados, 10% in Jamaica and between 13 and 14% in Dominica and St. Lucia.[8,9] In 2008, the United Nations (UN) recognized sickle cell anaemia, a form of SCD, as a public health concern. [10] The UN urged relevant parties to strengthen health systems and primary care delivery for sickle cell anaemia. [10] Since this declaration, many jurisdictions have recognized the need for SCD NBS in order to enable early identification and treatment of SCD affected infants. [10] Several studies have concluded that NBS for SCD and early treatment for SCD leads to reduced morbidity and mortality.[11] Notably, a study conducted in the Republic of Benin found that establishment of a NBS for SCD has led to a mortality

rate 10 times lower for children under 5 years of age.[12] In North America, SCD mortality for children under 5 years of age has been almost eliminated thanks to early identification and treatment from NBS.[13]

The Georgetown Public Hospital Corporation (GPHC) partnered with NSO to conduct a pilot study of NBS for CH and SCD in Guyana. Given the infrastructure challenges for establishing and delivering NBS in Guyana, partnering with an established NBS program could help overcome obstacles and help provide data that support the implementation of an NBS program, such as disease prevalence and the NBS test's positive predictive values in the Guyanese population.

Objectives

The objectives of this pilot NBS study were:

- To assess the feasibility of implementing a NBS program in Guyana for CH and HBG
- To assess the potential benefits of implementing a NBS program in Guyana for CH and HBG
- To report on screen positive rates for CH and HBG for the Guyanese population
- To report on HBG prevalence in Guyana based on NBS results and using the Hardy-Weinberg equilibrium (HWE)

Methods

Pilot study

Guyanese infants were eligible for this NBS pilot study if they were born at GPHC at a gestational age of 37 weeks or later and if they were not admitted to the neonatal intensive care unit. Written informed consent was obtained from the infant's mothers.

Shortly after birth (closer to 24 hours of life), a dried bloodspot (DBS) sample was collected with a heel prick and using a filter card. DBS samples were shipped weekly to NSO for analysis, with a

shipping turnaround time of three days. Samples were analysed for CH and SCD on the day of receipt by NSO, and the NBS results were sent from NSO to GPHC within approximately 14 days of samples receipt. NBS results were categorized as: screen positive (higher probability that the infant is affected by the disease and confirmatory diagnosis testing is needed), screen negative (low probability that the infant is affected by the disease and no follow up is needed), or unsatisfactory (the sample quality did not allow to perform the NBS test). Confirmatory testing and follow-up care were going to occur at GPHC, but infrastructure barriers kept GPHC from following up on screen positive infants.

CH

The screening test for CH was done using a human thyroid-stimulating hormone (hTSH) assay. The neonatal hTSH assay is a solid phase, two site fluoroimmunometric assay based on the direct sandwich technique. Specimens containing hTSH are reacted with anti- hTSH IgG, coated on the wells of the microtitre plates, and europium-labelled anti- hTSH IgG (Eu tracer). The wash buffer elutes hTSH from the DBS. The enhancement solution dissociates the europium ions from the labeled antibody where they form highly fluorescent chelates. The fluorescence measured in each well is proportional to the concentration of hTSH in the sample. If the initial hTSH level in a sample was found to be greater than or equal to 13 µU/mL of blood, a confirmatory hTSH measurement was made using the same technique outlined above. If the confirmatory hTSH level was found to be greater than or equal to 17 µU/mL of blood, the newborn was considered screen positive for CH. Otherwise, the patient is said to be screen-negative for CH. Mean hTSH levels between the Guyanese sample and the Ontarian population were compared using Student's T test with an alpha of 0.05.

SCD

The screening test for SCD was performed with a cation-exchange high-performance liquid chromatography (HPLC) used to separate the different variants of hemoglobin present in a DBS

elutes (reconstituted DBS). Using a two dual-piston pump, the ionic strength of the buffer is increased encouraging the more strongly retained hemoglobin to elute from the column. Each hemoglobin has a characteristic retention time. The instrument quantifies the amount of hemoglobin variants present in the sample. These include adult hemoglobins (A, A2) which are the most common types in the normal population; fetal hemoglobins (F, F1) and minor hemoglobins (E, S, C). Together, the quantitative hemoglobin results (in the form of peaks in the chromatogram) are interpreted by a clinician who assigns the chromatogram a single *hemoglobin pattern* determination. The hemoglobin pattern determination is used to determine if the newborn is said to be screen negative or screen positive for SCD. This NBS tests allows for identification of carriers of SCD.

Research Ethics

This study was reviewed and approved by the Guyana Ministry of Public Health Institutional Review Board (IRB) and by the Children's Hospital of Eastern Ontario Research Ethics Board.

Patient and Public Involvement

Patients were recruited as the sample population. Newborn babies were tested after mothers were given a written document and a brief discussion of the purpose of the research, the risks and benefits of being tested and the potential complications from a heel prick DBS sample collection. A written consent form was provided to mothers for signature. Some mothers requested that the fathers consent as well. Only babies whose parents consented were included in the study. Data was coded with a unique identifier.

Funding

This project was funded by Guyana Bank for Trading and Industry (GBTI) through a public-private partnership. Funds were used to purchase supplies for sample collection and for testing. Georgetown Public Hospital Corporation (GPHC) paid for the shipment of samples to NSO weekly and provided human resources for sample collection.

Results

Pilot experience and feasibility

The pilot was conducted from June 6th, 2016 to September 22nd, 2017. GPHC recruited 2,294 mothers/infants. All samples were successfully shipped to NSO. Diagnostic data for the screen positive infants were not obtained since telephone numbers provided were unreachable and mothers failed to bring in the child so this report is solely on the screen positive rate for CH and SCD, and on the estimated prevalence of SCD assuming HWE. Of note, one mother followed up and her child was confirmed as SC.

CH

Of the 2,294 received samples, 256 were excluded of CH NBS testing as they were determined unsatisfactory for testing. The most common reason for exclusion was a non-sufficient blood quantity in the sample (n=218). The screen positive rate for CH in our sample was 0.0% (0/2,038 infants). Mean TSH levels in Guyanese samples (1.7 µU/mL blood) was noticed to be significantly different than in the Ontario population (4.3 µU/mL blood) ($p < 0.05$).

SCD

Of the 2,294 received samples, 255 were excluded of SCD NBS testing as they were determined unsatisfactory for testing. The most common reason for exclusion was a non-sufficient blood quantity in the sample (n=218). The screen positive rate for SCD in our sample was 0.3% (7/2,039 patients), and the carrier rate was 8.41% (172/2,039patients). As we were unable to obtain follow-up diagnostic data for the screen positive infants, the true positive rate and the positive predictive value of this SCD NBS test in the Guyanese population could not be ascertained. However, as this NBS tests identifies SCD-affected infants as well as carriers, the allele frequency for SCD in the study sample and estimate of SCD prevalence in the Guyanese population using the HWE, assuming sample is representative of the population in terms of allele frequency could be deduced. The HWE is commonly used to estimate the frequency of a phenotype in a population, for example a recessive condition like SCD, because sample sizes in cross-sectional studies are usually too small to allow for a

precise estimate. [14,15]Table 1 presents the genotype and S allele frequencies in the study sample, the S allele frequency being represented by equation 1:

(1) S allele frequency = (number of carriers) + (2 x (number of screen positive) / (number of infants screened x 2)

Table 1: SCD genotype and S allele frequencies in the study sample

AA frequency – screen negative (%)	AS frequency – carriers (%)	SS frequency – screen positive	S allele frequency
51,35	9,07	0,34	0,049

Using the HWE, the SCD frequency is (S allele frequency) ^{2} = 0,49 ^{2} = 0.002.

Since Guyana has a population of approximately 746,955 (according to a census conducted in 2012 conducted by the Bureau of Statistics of Guyana) [16] it can be estimated that the SCD prevalence in is 1,494. Further, the Bureau of statistics estimated the projected births to be between 13,963 and 15,864 between 2000 and 2025 in Guyana. [16]Using the projected estimate birth value for 2020, 15,126 births, it can be estimated that the birth prevalence of SCD in Guyana will be approximately 30 births per year (0.2%).

CH and SCD as NBS candidate diseases in Guyana as per Wilson-Jungner criteria

To further assess the suitability of CH and SCD NBS as a public health program in Guyana, mapping of Guyana's experience was done onto the Wilson-Jungner criteria. These criteria were established in 1968 in a report for the World Health Organization[17]and have been developed as an evaluation framework to determine the eligibility of a given condition and its test for a screening program. The Wilson-Jungner principle have been widely used as a checklist to establish NBS programs.[18]The principles acknowledge the technical characteristics of a given NBS test as well the significance of political, economic, social and health issues of an NBS test or program[17]. Table 2 presents the 10 Wilson-Jungner principles and whether each principle was met in this pilot study. Seven out of the

ten principles were met in the study, and the remaining two could not be determined. based on the observations.

Table 2: Wilson-Jungner principles

Principle	Principle met?
1. The condition sought should be an important health problem.	Yes
2. There should be an accepted treatment for patients with recognized diseases	Yes
3. Facilities for diagnosis and treatment should be available	Unknown yet – unable to be assessed in this pilot
4. There should be a recognizable latent or early symptomatic phase	Yes
5. There should be a suitable test or examination	Yes
6. The test should be acceptable to the population	Yes
7. The natural history of the condition, including development from latent to declared disease, should be understood	Yes
8. There should be an agreed policy on whom to treat as patients	Draft document awaiting final approval by Ministry of Public Health, Guyana
9. The cost of case-finding (including diagnosis and treatment of patients diagnosed should be economically balanced in relation to possible expenditure on medical care as a whole	Yes
10. Case-finding should be a continuing process and not a “once and for all” project	Yes–policy brief for implementation of NBS was recently presented to the Pan American Heath Organization (PAHO) for implementation of routine NBS in 2020.

Discussion

A pilot study of NBS in Guyana was conducted in which a remote laboratory performed the screening tests. The pilot has shed light on the feasibility and potential benefits of NBS for CH and SCD in the Guyanese population. This pilot study also provided an estimate of the SCD prevalence and birth prevalence in Guyana.

There were no screen positive infants for CH in the sample population, however the relatively high CH prevalence overall and in neighbouring jurisdictions suggests that some of the infants born in Guyana could be affected by CH and benefit from NBS. [2] Moreover, NBS for CH had proven to be effective in reducing mortality and morbidity, and the treatment is inexpensive, making the justification for establishing NBS compelling. [1] Currently, children are tested for hypothyroidism if there is a high index of suspicion based on history and physical examination (such as developmental delay). Late diagnosis and intervention have adverse prognostic factors for these children. Further research is needed to look into the potential causes of the mean TSH levels difference between Guyana and Ontario, and whether TSH cut-offs for CH NBS in Guyana have to be adjusted accordingly. It would notably be important to determine whether the shipment time and conditions (temperature, humidity, etc.) from Guyana to Ontario, Canada affects TSH levels in the DBS, or if mean TSH levels are different in the Guyanese population due to other factors specific to this population. It is possible that some infants in the study sample are in fact affected by CH, and diagnosis could not have been confirmed due to lack of follow up data. Confirming diagnosis would further help determining if the TSH cut-off for CH NBS tests in Guyana should be different than the one used in Ontario by NSO.

The sample's screen positive rate for SCD (0.3%), as well as the estimated SCD allele frequency and birth prevalence of SCD in Guyana assuming HWE (0.2%) are lower than what has been reported in some Caribbean jurisdictions. The SCD birth prevalence has been estimated to be of 2.7% in Aruba and 6.8 % in St. Maarten, and the SCD allele frequency has been reported to be ,7% in Aruba, 7.3% in St. Maarten, 7% in Barbados, 10% in Jamaica and between 13 and 14% in Dominica and St. Lucia. [6,7] This discrepancy could be due to a small sample, insufficient statistical power and insufficient external validity of the study sample. To our knowledge, this is the first report of an estimated prevalence and birth prevalence of SCD in Guyana, and further research is needed to clarify these findings. Despite being relatively low, the estimated birth prevalence suggests that about 30

newborns per year in Guyana are affected by SCD, which indicates that Guyana could benefit from SCD NBS to reduce the condition's mortality and morbidity. Guyana recently concluded a draft document for the management of SCD and Thalassemia. There is a SCD clinic that is conducted weekly at GPHC that provides specialized care for SCD children. The Guyana Sickle Cell Association also provides support for these patients in terms of educational resources and coping strategies. Of recent, there are two hematology fellows at GPHC who are capable of providing specialised care for these patients.

The eligibility of CH and SCD NBS in Guyana is further supported by our assessment of this pilot's experience using the Wilson-Jungner principles. Only two of the ten principles have not been met in this study. These two unmet principles were out of the study's scope and could be established in future work. Principle three (3), *Facilities for diagnosis and treatment should be available*, and Principle eight (8), *There should be an agreed policy on whom to treat as patients*, could be met through some policy work in Guyana.

Overall, our experience suggests that NBS for CH and SCD in Guyana could be beneficial, and that it is feasible to establish a program by shipping DBS samples to a remote NBS lab for analysis. Our experience also suggests that remote NBS could be an avenue towards establishing NBS programs in countries with no existing NBS infrastructure.

Future work should focus on conducting larger pilots that would allow more diagnostic data to be collected, which in turn would allow a more accurate estimate of positive predictive values of the NBS tests and to a better estimation of the mortality and morbidity reduction in Guyana thanks to NBS. Additionally, these finding could be used to inform diagnosis and treatment guidelines for Guyanese people.

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Abstract

Introduction: Newborn screening (NBS) is a test done shortly after birth to detect conditions that cause severe health problems if not treated early. Approximately 71% of babies worldwide are born in a jurisdiction that does not have an established NBS program. Guyana currently has no NBS program and has established a partnership with Newborn Screening Ontario (NSO) to initiate screening.

Objectives: to assess the feasibility of implementing a NBS program in Guyana for congenital hypothyroidism (CH) and hemoglobinopathies (HBG) and to report on screen positive rates and prevalence (Hardy-Weinberg equilibrium (HWE) for CH and HBG.

Methods: Term, healthy Guyanese infants were tested (with consent) using heel prick dried blood spots (DBS) shortly after birth (closer to 24 hours of life). DBS samples were analysed at NSO. Screening test for CH was done using a human thyroid-stimulating hormone (hTSH) assay. Mean hTSH levels between the Guyanese sample and the Ontarian population were compared using

Student's T test with an alpha of 0.05. Screening test for SCD was performed with a cation-exchange high-performance liquid chromatography (HPLC).

Results: The pilot was conducted from June 6th, 2016 to September 22nd, 2017. GPHC recruited 2,294 mothers/infants. Screen positive rate for CH in our sample was 0.0% (0/2,038 infants). Mean TSH levels in Guyanese samples (1.7 µU/mL blood) was noticed to be significantly different than in the Ontario population (4.3 µU/mL blood) ($p < 0.05$). Screen positive rate for SCD in our sample was 0.3% (7/2,039 patients), and the carrier rate was 8.41% (172/2,039patients). Using the HWE, the SCD frequency is (S allele frequency) $^2 = 0{,}49\ ^2 = 0.002$.

Conclusion: NBS for CH and SCD in Guyana could be beneficial. Future work should focus on conducting larger pilots which could be used to inform diagnosis and treatment guidelines for Guyanese people.

Article Summary

Strengths and limitations of this study

- This is the first study on newborn screening to be conducted in Guyana. It provides baseline data for sickle cell anemia and for thyroid hormone levels for newborn babies in Guyana.
- Data can be used by policy makers to justify future work in newborn screening in Guyana.
- Limitations include lack of previous baseline data for comparison and interpretation especially with baseline thyroid hormone levels. Relatively small sample size and single centre study due to limited of resources. Difficulty with follow- up of borderline or positive results due to geographic and constraints

Introduction

Newborn screening (NBS) is a screening test done shortly after birth to detect conditions that cause severe health problems if not treated early.[1]NBS programs have been existing for over 50 years in developed countries.[2]It allows clinical providers to identify infants at high risk for those conditions, provide diagnostic testing and initiate treatment to avoid severe outcome and to prevent death.[3]Conditions included in NBS need to have a relatively high prevalence and their testing and treatment need to be simple, inexpensive and effective.[2]While the number of conditions included in NBS panels varies across jurisdictions, the most advanced laboratory settings give NBS programs the capability for screening for over 50 conditions.[3]

NBS programs in high income countries have made significant advances, thanks to the advent of centralized laboratory services and advanced analytical methods, such as tandem mass spectrometry, which has increase the NBS tests' sensitivity and rapidity.[4]In lower- and middle-income jurisdictions, barriers such as lack of resources and infrastructures have limited the development of NBS programs.[4]Approximately 71% of babies worldwide are born in a jurisdiction that does not have an established NBS program.[2] As such, the majority of infants born worldwide with diseases for which NBS exists do not get diagnosed and treated early. This leads to significant mortality, morbidity and economic burden of NBS-eligible diseases in jurisdictions where NBS is not developed. [2]

Guyana currently has no NBS program and has established a partnership with Newborn Screening Ontario (NSO) to initiate screening. NSO was established in 2006 and coordinates NBS for the province of Ontario, Canada. NSO screens over 140,000 newborns per year for 29 targeted diseases by collecting and analyzing dried bloodspot (DBS) samples in the first days of life. Congenital hypothyroidism (CH) and sickle cell disease (SCD) are two relatively common conditions, that are good candidates for NBS and offered by NSO. In the Guyanese population, the prevalence of CH and

SCD are unknown but there is evidence they have a significant public health burden, making these two conditions good candidates for a NBS pilot in Guyana.

CH is an endocrine condition that can result in growth deficiency and severe intellectual disability if left untreated.[1]It is one of the most common preventable cause of intellectual disability with an incidence in newborns between 1:2000 and 1:4000, and as high as 1:600 newborns in iodine deficient areas. [1]CH is most often permanent and caused by an abnormality in thyroid gland development or a defect in thyroid hormonogenesis. [5]In some cases, the condition is transient, in which case it can be due to the transplacental passage of maternal medication, maternal blocking antibodies, iodine deficiency or excess. [5]Treatment for CH is effective, inexpensive and easy to manage for families.[1]Infants diagnosed with CH near birth can start treatment and avoid any growth delay or intellectual disability and develop normally.[1]However, when CH patients are identified later, treatment does not suffice to eliminate developmental and growth delays resulting from CH, which underlines the importance of NBS for CH. [1]CH was added to NBS programs starting in the mid-1970s, and has shown to be effective in reducing morbidity, mortality and the economic burden of CH: the jurisdictions screening for CH have eliminated neuro-developmental impairments resulting from CH.[2]Since establishing NBS for CH, Cuba, Puerto Rico and the US Virgin Island have been screening more than 95% of their population for CH and reported a birth incidence ranging from 1: 3,600 and 1: 9,526. [2]In Brazil, the CH prevalence has been reported as 1: 2,259. [2]The CH prevalence in Guyana remains unknown.

In Guyana, SCD was found to be among the top leading causes for admission to pediatric wards. During a one-year study, it accounted for 102 of 1380 admissions.[6]SCD is a group of recessive inherited conditions that affects the formation of haemoglobin and the functioning of red blood cells. The gene mutations responsible for SCD are most common in populations of African, South Asian, Middle Eastern and Mediterranean origins.[7]SCD is one of the most common genetic conditions worldwide, with a birth incidence estimated at 24,900 cases per year. [7]

SCD is a global health public concern, a survey conducted by the World Health Organization (WHO) reported that haemoglobin disorders such as SCD are a public health concern for 71% of the 229 countries surveyed, who represent 89% of births worldwide. [7]

While the prevalence of SCD in Guyana in unknown, data is available for some Caribbean countries through NBS. Jamaica has the highest prevalence of SCD at 0.65%, followed by Grenada (0.63%), Haiti (0.58%) and Tobago (0.57%). Trait prevalence was highest in Jamaica at 14% and St. Vincent and Grenadines (15%), followed by St. Lucia (13.8%), Haiti (13.5%) and Tobago (13.2%). [8]

SCD patients experience a wide range of adverse outcomes, including, but not limited to: anaemia, stroke, ischemic organ damage, pain crises, chronic respiratory disease, recurrent infections, and death. [9]. In 2008, the United Nations (UN) recognized sickle cell anaemia, a form of SCD, as a public health concern and urged relevant parties to strengthen health systems and primary care delivery for sickle cell anaemia. [10] Since this declaration, many jurisdictions have recognized the need for SCD NBS in order to enable early identification and treatment of SCD affected infants. [10] Several studies have concluded that NBS for SCD and early treatment for SCD leads to reduced morbidity and mortality. Notably, a study conducted in the Republic of Benin found that establishment of a NBS for SCD has led to a mortality rate 10 times lower for children under 5 years of age.[11] In North America, SCD mortality for children under 5 years of age has been almost eliminated thanks to early identification and treatment from NBS.[12]

The Georgetown Public Hospital Corporation (GPHC) partnered with NSO to conduct a pilot study of NBS for CH and SCD in Guyana. Given the infrastructure challenges for establishing and delivering NBS in Guyana, partnering with an established NBS program could help overcome obstacles and help provide data that support the implementation of a NBS program, such as disease prevalence and the NBS test's positive predictive values in the Guyanese population.

Objectives

The objectives of this pilot NBS study were:

- To assess the feasibility of implementing a NBS program in Guyana for CH and HBG
- To assess the potential benefits of implementing a NBS program in Guyana for CH and HBG
- To report on screen positive rates for CH and HBG (sickle cell disease and traits) for the Guyanese population
- To report on HBG prevalence in Guyana based on NBS results and using the Hardy-Weinberg equilibrium (HWE)

Methods

Study Design

This pilot project was a prospective descriptive study at done at GPHC from June 6th, 2016 to September 22nd, 2017.

Inclusion criteria

Term, healthy babies (gestational age of 37 weeks or later) born at GPHC during the study period whose mothers provided written consent after being briefed on the study.

Exclusion criteria

Babies with sepsis, and those admitted to the neonatal intensive care unit. Sick newborns can have abnormal TSH due to their illness which may not be indicative of CH.

Sample size

This was calculated for all babies born during the study period (1 year), based on duration of funding, representing approximately 3250 neonates. Guyana has an average of 15,000 live births per year with 22% being born at GPHC. It was not feasible to include other rural hospitals in this pilot study.

Sample Collection and handling

Shortly after birth (closer to 24 hours of life), a dried bloodspot (DBS) sample was collected by trained laboratory technicians via heel prick and using a filter card. These samples were stored at the central laboratory at GPHC according to standard protocols for storing DBS. DBS filter cards were air shipped (at room temperature) weekly to NSO for analysis, with a shipping turnaround time of three days.

Analysis

Samples were analysed for CH and SCD on the day of receipt by NSO, and the NBS results were sent from NSO to GPHC within approximately 14 days of samples receipt. NBS results were categorized as: screen positive (higher probability that the infant is affected by the disease and confirmatory diagnosis testing is needed), screen negative (low probability that the infant is affected by the disease and no follow up is needed), or unsatisfactory (the sample quality did not allow to perform the NBS test). Confirmatory testing and follow-up care were planned at GPHC.

CH

The screening test for CH was done using a human thyroid-stimulating hormone (hTSH) assay. The neonatal hTSH assay is a solid phase, two site fluoroimmunometric assay based on the direct sandwich technique. Specimens containing hTSH are reacted with anti- hTSH IgG, coated on the wells of the microtitre plates, and europium-labelled anti- hTSH IgG (Eu tracer). The wash buffer elutes hTSH from the DBS. The enhancement solution dissociates the europium ions from the labeled antibody where they form highly fluorescent chelates. The fluorescence measured in each well is proportional to the concentration of hTSH in the sample. If the initial hTSH level in a sample was found to be greater than or equal to 13 µU/mL of blood, a confirmatory hTSH measurement was made using the same technique outlined above. If the confirmatory hTSH level was found to be

greater than or equal to 17 µU/mL of blood, the newborn was considered screen positive for CH. Otherwise, the patient is said to be screen-negative for CH. Mean hTSH levels between the Guyanese sample and the Ontarian population were compared using Student's T test with an alpha of 0.05.

SCD

The screening test for SCD was performed with a cation-exchange high-performance liquid chromatography (HPLC) used to separate the different variants of hemoglobin present in a DBS elutes (reconstituted DBS). Using a two dual-piston pump, the ionic strength of the buffer is increased encouraging the more strongly retained hemoglobin to elute from the column. Each hemoglobin has a characteristic retention time. The instrument quantifies the amount of hemoglobin variants present in the sample. These include adult hemoglobins (A, A2) which are the most common types in the normal population; fetal hemoglobins (F, F1) and minor hemoglobins (E, S, C). Together, the quantitative hemoglobin results (in the form of peaks in the chromatogram) are interpreted by a clinician who assigns the chromatogram a single *hemoglobin pattern* determination. The hemoglobin pattern determination is used to determine if the newborn is said to be screen negative or screen positive for SCD. This NBS tests allows for identification of carriers of SCD.

S allele frequency was calculated using the below equation (equation 1)

(1) S allele frequency = (number of carriers) + (2 x (number of screen positive) / (number of infants screened x 2)

SCD prevalence in Guyana was estimated using a population of approximately 746,955 (according to a census conducted in 2012 conducted by the Bureau of Statistics of Guyana) [13]. The Bureau of Statistics estimated projected births be between 13,963 and 15,864 between 2000 and 2025 in Guyana was used to calculate the birth prevalence of SCD. [13]

Outcome measures

Primary outcomes were to report on screen positive rates for CH and HBG for the Guyanese population using Hardy-Weinberg equilibrium (HWE) and percent positivity rates. The HWE is commonly used to estimate the frequency of a phenotype in a population, for example a recessive condition like SCD, because sample sizes in cross-sectional studies are usually too small to allow for a precise estimate. [14,15]. For CH, mean hTSH levels and mean difference between the Guyanese sample and the Ontarian population were compared using Student's T test with an alpha of 0.05.

Secondary outcome was to assess the feasibility of CH and SCD NBS as a public health program in Guyana using *Wilson-Jungner criteria* as the outcome measure.

Table 1: Wilson-Jungner principles [16]

Principle	Principle met?
1. The condition sought should be an important health problem.	Yes
2. There should be an accepted treatment for patients with recognized diseases	Yes
3. Facilities for diagnosis and treatment should be available	Available but was not assessed in this pilot. GPHC can diagnose and treat SCD and CH.
4. There should be a recognizable latent or early symptomatic phase	Yes
5. There should be a suitable test or examination	Yes
6. The test should be acceptable to the population	Yes
7. The natural history of the condition, including development from latent to declared disease, should be understood	Yes
8. There should be an agreed policy on whom to treat as patients	Draft document awaiting final approval by Ministry of Public Health, Guyana
9. The cost of case-finding (including diagnosis and treatment of patients diagnosed should be economically balanced in relation to possible expenditure on medical care as a whole	Yes
10. Case-finding should be a continuing process and not a "once and for all" project	Yes–policy brief for implementation of NBS was presented to the Pan American Heath Organization (PAHO) Guyana office in 2020 for implementation of routine NBS. No

	further work in on this due to COVID-pandemic.

Research Ethics

This study was reviewed and approved by the Guyana Ministry of Public Health Institutional Review Board (IRB #225) and by the Children's Hospital of Eastern Ontario Research Ethics Board (REB Protocol No: 17/210X).

Patient and Public Involvement

Patients were recruited as the sample population. Newborn babies were tested after mothers were given a written document and a brief discussion of the purpose of the research, the risks and benefits of being tested and the potential complications from a heel prick DBS sample collection. A written consent form was provided to mothers for signature. Some mothers requested that the fathers' consent as well. Only babies whose parents consented were included in the study. Data was coded with a unique identifier.

Results

Pilot experience and feasibility

The pilot was conducted from June 6th, 2016 to September 22nd, 2017. GPHC recruited 2,294 mothers/infants. There were no reported harms to babies who were tested. All samples were successfully shipped to NSO. Diagnostic data for all of the screen positive infants were not obtained since telephone numbers provided were unreachable and mothers failed to bring in their child, so this report is solely on the screen positive rate for CH and SCD, and on the estimated prevalence of SCD assuming HWE. Of note, one mother followed up and her child was confirmed as SC.

Flow diagram summarizing sample collection and analysis for CH and SCD

CH

Of the 2,294 received samples, 256 were excluded of CH NBS testing as they were determined unsatisfactory for testing. The most common reason for exclusion was a non-sufficient blood quantity in the sample (n=218). The screen positive rate for CH in our sample was 0.0% (0/2,038 infants). Mean TSH levels in Guyanese samples (1.7 µU/mL blood) was significantly different ($p < 0.05$) than in the Ontario population (4.3 µU/mL blood).

SCD

Of the 2,294 received samples, 255 were excluded of SCD NBS testing as they were determined unsatisfactory for testing. The most common reason for exclusion was a non-sufficient blood quantity in the sample (n=218). The screen positive rate for SCD in our sample was 0.3% (7/2,039 patients), and the carrier rate was 8.41% (172/2,039 patients). As we were unable to obtain follow-up diagnostic data for all of the screen positive infants, the true positive rate and the positive predictive value of this SCD NBS test in the Guyanese population could not be ascertained. However, as this NBS tests identifies SCD-affected infants as well as carriers, the allele frequency for SCD in the study sample and estimate of SCD prevalence in the Guyanese population using the HWE, assuming sample is representative of the population in terms of allele frequency could be deduced. Table 1 presents the genotype and S allele frequencies in the study sample, the S allele frequency being represented by equation 1 in methods section:

Table 2: SCD genotype and S allele frequencies in the study sample

AA frequency – screen negative (%)	AS frequency – carriers (%)	SS frequency – screen positive	S allele frequency
51,35	9,07	0,34	0,049

Using the HWE, the SCD frequency is (S allele frequency) ^{2} = 0,49 ^{2} = 0.002.

Using the projected estimate birth value for 2020 of 15,126 births, it can be estimated that the birth prevalence of SCD in Guyana is approximately 30 births per year (0.2%).

CH and SCD as NBS candidate diseases in Guyana as per Wilson-Jungner criteria

To further assess the suitability of CH and SCD NBS as a public health program in Guyana, mapping of Guyana's experience was done onto the Wilson-Jungner criteria. These criteria were established in 1968 in a report for the World Health Organization[17]and have been developed as an evaluation framework to determine the eligibility of a given condition and its test for a screening program. The Wilson-Jungner principle have been widely used as a checklist to establish NBS programs.[17]The principles acknowledge the technical characteristics of a given NBS test as well the significance of political, economic, social and health issues of an NBS test or program[16]. Table 2 presents the 10 Wilson-Jungner principles and whether each principle was met in this pilot study. Seven out of the ten principles were met in the study. Principle 3 was not assessed in this pilot but is met locally. Principles 8 and 10 were being addressed prior to the pandemic and is currently on hold due to prioritization of resources, see table 1 in methods section.

Discussion

A pilot study of NBS in Guyana was conducted in which a remote laboratory performed the screening tests. The pilot has shed light on the feasibility and potential benefits of NBS for CH and SCD in the Guyanese population. This pilot study also provided an estimate of the SCD prevalence and birth prevalence in Guyana.

There were no screen positive infants for CH in the sample population, but the researchers are familiar with cases of CH in Guyana. Small sample population of 2294 was probably responsible for zero positivity for CH as incidence ranges from 1:2000 to 1:4000. At least 4000-5000 babies will need screening for one positive sample. [1] Currently, children are tested for hypothyroidism in Guyana if

there is a high index of suspicion such as developmental delay. Late diagnosis and intervention have adverse prognostic factors for these children. Further research is needed to determine the prevalence of CH in newborns and the potential causes of the mean TSH levels difference between Guyana and Ontario, and whether TSH cut-offs for CH NBS in Guyana have to be adjusted accordingly. It would notably be important to determine whether the shipment time and conditions (temperature, humidity, etc.) from Guyana to Ontario, Canada affects TSH levels in the DBS, or if mean TSH levels are different in the Guyanese population due to other factors specific to this population. It is possible that some infants in the study sample are in fact affected by CH with TSH levels higher than the mean TSH level for the sample population. Diagnosis of CH could not have been confirmed due to lack of follow up data. Confirming diagnosis would further help determining if the TSH cut-off for CH NBS tests in Guyana should be different than the one used in Ontario by NSO. Currently, we are unable to comment on recommending CH as a priority NBS in Guyana.

The sample's screen positive rate for SCD (0.3%), as well as the estimated SCD allele frequency and birth prevalence of SCD in Guyana assuming HWE (0.2%) are lower than what has been reported in some Caribbean jurisdictions.[8] This may be due to small sample size, insufficient statistical power and insufficient external validity of the study sample, and centralized testing at GPHC without having a good representation of the rural areas in the sample population as SCD is higher in Afro-Caribbean people. To our knowledge, this is the first report of an estimated prevalence and birth prevalence of SCD in Guyana, and further research is needed to clarify these findings. The estimated birth prevalence from this study suggests that about 30 newborns per year in Guyana are affected by SCD, which indicates that Guyana may benefit from SCD NBS. Guyana recently concluded a draft document for the management of SCD and Thalassemia and there is an increase in speciality service for this population.

The eligibility of CH and SCD NBS in Guyana is further supported by our assessment of this pilot's experience using the Wilson-Jungner principles. Only three of the ten principles have not been met in this study. These three unmet principles were out of the study's scope and could be established in future work. Principle three (3), *Facilities for diagnosis and treatment should be available*, Principle eight (8), *There should be an agreed policy on whom to treat as patients*, and Principle ten (10), *Case-finding should be a continuing process and not a "once and for all" project,* could be met through some policy work in Guyana. The first author has developed a policy brief for the implementation of NBS for HGB in Guyana under the guidance of Pan American Health Organization (PAHO).

Overall, our experience suggests that NBS for CH and SCD in Guyana could be beneficial, and that it is feasible to establish a program by shipping DBS samples to a remote NBS lab for analysis while Guyana builds capacity for local testing.

Future work should focus on conducting larger pilots that would allow more diagnostic data to be collected, which in turn would allow a more accurate estimate of positive predictive values of the NBS tests and a better estimation of the mortality and morbidity reduction in Guyana if NBS is implemented. Additionally, these finding could be used to inform diagnosis and treatment guidelines for Guyanese people.

Limitations

Lack of access to testing facilities in Guyana was a major hinderance to the start of this project until NSO became involved. Only children born centrally were included in the study due to lack of resources and access to rural areas. Sample population may not be a true representation of the Guyanese population distribution. There were no positive cases of CH due to small sample size as CH prevalence ranges from 1:2000 to 1:9000. Larger studies are needed to establish prevalence of CH in newborns in Guyana, limited funding did not permit a larger study at this time.

Sample rejection was 11%, this was due to lack of familiarity of DBS as a routine method of sample collection. Technicians were trained for DBS but skill set varied among technicians. This further reduced our sample size for analysis.

We were unable to follow up most of the screen positive cases for SCD despite many attempts as these cases were all from the rural areas and contact information was not reliable.

Contributorship statement

Bibi A. Alladin, Pheona Mohamed-Rambaran, Vijay Grey and Andrea Hunter developed the proposal.

Bibi A. Alladin and Pheona Mohamed-Rambaran provided resources and supervision for sample collection, shipment and follow up of results locally, including reporting and patient contact.

Bibi A. Alladin secured funding, completed the final manuscript and article submission.

Pranesh Chakraborty, Matthew Henderson, Jennifer Milburn, Laure A. Tessier contributed to the methodology, provided resources for analyzing samples, interpretation of the results and assisted with compilation of the manuscript.

Competing interests

There were no competing interests for any of the authors.

Funding

Grant number: Not applicable

This project was funded by Guyana Bank for Trading and Industry (GBTI) through a public-private partnership. Funds were used to purchase supplies for sample collection and for testing.

Georgetown Public Hospital Corporation (GPHC) paid for the shipment of samples to NSO weekly and provided human resources for sample collection.

Data sharing statement

Extra data is available and can be accessed by emailing pheonar@yahoo.com.

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Dr. Jennifer McKnight provided information on NBS in the Caribbean and has volunteered to assist with implementation of NBS in Guyana.

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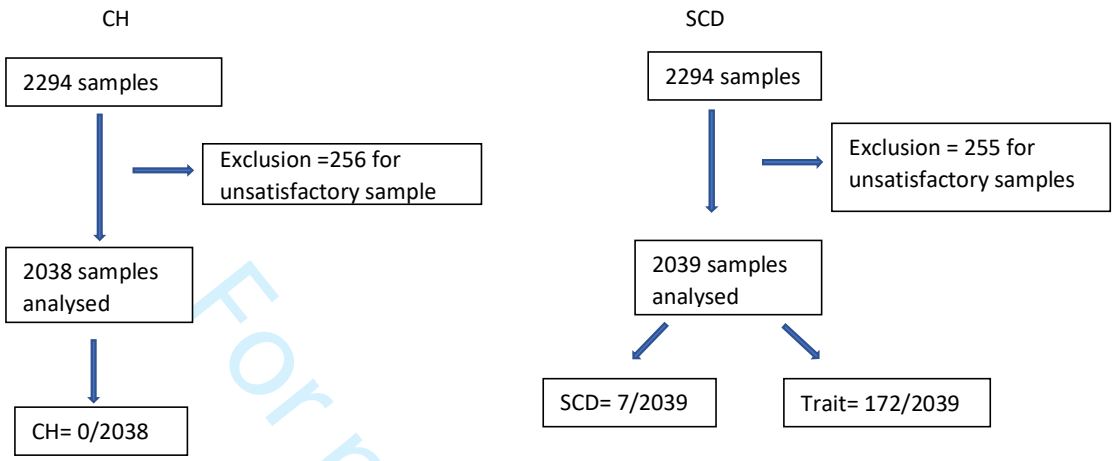
Flow diagram summarizing sample collection and analysis for CH and SCD



CONSORT 2010 checklist of information to include when reporting a pilot or feasibility trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a pilot or feasibility randomised trial in the title	1
	1b	Structured summary of pilot trial design, methods, results, and conclusions (for specific guidance see CONSORT abstract extension for pilot trials)	1
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale for future definitive trial, and reasons for randomised pilot trial	2-5
	2b	Specific objectives or research questions for pilot trial	5
Methods			
Trial design	3a	Description of pilot trial design (such as parallel, factorial) including allocation ratio	
	3b	Important changes to methods after pilot trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	5-6
	4b	Settings and locations where the data were collected	5-6
	4c	How participants were identified and consented	6,9,10
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	
Outcomes	6a	Completely defined prespecified assessments or measurements to address each pilot trial objective specified in 2b, including how and when they were assessed	8
	6b	Any changes to pilot trial assessments or measurements after the pilot trial commenced, with reasons	
	6c	If applicable, prespecified criteria used to judge whether, or how, to proceed with future definitive trial	
Sample size	7a	Rationale for numbers in the pilot trial	
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	
	8b	Type of randomisation(s); details of any restriction (such as blocking and block size)	
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	

Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12	Methods used to address each pilot trial objective whether qualitative or quantitative	6-8
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were approached and/or assessed for eligibility, randomly assigned, received intended treatment, and were assessed for each objective	10
	13b	For each group, losses and exclusions after randomisation, together with reasons	
Recruitment	14a	Dates defining the periods of recruitment and follow-up	
	14b	Why the pilot trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	
Numbers analysed	16	For each objective, number of participants (denominator) included in each analysis. If relevant, these numbers should be by randomised group	10
Outcomes and estimation	17	For each objective, results including expressions of uncertainty (such as 95% confidence interval) for any estimates. If relevant, these results should be by randomised group	10-12
Ancillary analyses	18	Results of any other analyses performed that could be used to inform the future definitive trial	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	10
	19a	If relevant, other important unintended consequences	
Discussion			
Limitations	20	Pilot trial limitations, addressing sources of potential bias and remaining uncertainty about feasibility	14
Generalisability	21	Generalisability (applicability) of pilot trial methods and findings to future definitive trial and other studies	13-14
Interpretation	22	Interpretation consistent with pilot trial objectives and findings, balancing potential benefits and harms, and considering other relevant evidence	12-14
	22a	Implications for progression from pilot to future definitive trial, including any proposed amendments	
Other information			
Registration	23	Registration number for pilot trial and name of trial registry	
Protocol	24	Where the pilot trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	15
	26	Ethical approval or approval by research review committee, confirmed with reference number	9

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*We strongly recommend reading this statement in conjunction with the CONSORT 2010, extension to randomised pilot and feasibility trials, Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

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A pilot project to determine the feasibility of implementing newborn screening for sickle cell anemia and congenital hypothyroidism in Guyana

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Abstract

Introduction: Newborn screening (NBS) is a test done shortly after birth to detect conditions that cause severe health problems if not treated early. Approximately 71% of babies worldwide are born in a jurisdiction that does not have an established NBS program. Guyana currently has no NBS program and has established a partnership with Newborn Screening Ontario (NSO) to initiate screening.

Objectives: to assess the feasibility of implementing a NBS program in Guyana for congenital hypothyroidism (CH) and hemoglobinopathies (HBG) and to report on screen positive rates and prevalence (Hardy-Weinberg equilibrium (HWE) for CH and HBG.

Methods: Term, healthy Guyanese infants were tested (with consent) using heel prick dried blood spots (DBS) shortly after birth (closer to 24 hours of life). DBS samples were analysed at NSO. Screening test for CH was done using a human thyroid-stimulating hormone (hTSH) assay. Mean hTSH levels between the Guyanese sample and the Ontarian population were compared using

Student's T test with an alpha of 0.05. Screening test for SCD was performed with a cation-exchange high-performance liquid chromatography (HPLC).

Results: The pilot was conducted from June 6th, 2016, to September 22nd, 2017. GPHC recruited 2,294 mothers/infants. Screen positive rate for CH in our sample was 0.0% (0/2,038 infants). Mean TSH levels in Guyanese samples (1.7 µU/mL blood) was noticed to be significantly different than in the Ontario population (4.3 µU/mL blood) ($p < 0.05$). Screen positive rate for SCD in our sample was 0.3% (7/2,039 patients), and the carrier rate was 8.41% (172/2,039patients). Using the HWE, the SCD frequency is (S allele frequency) ^{2} = 0,49 ^{2} = 0.002.

Conclusion: NBS for CH and SCD in Guyana could be beneficial. Future work should focus on conducting larger pilots which could be used to inform diagnosis and treatment guidelines for Guyanese people.

Article Summary

Strengths and limitations of this study

- This first-time study on newborn screening in Guyana provides baseline data on sickle cell anemia and thyroid hormone levels.
- The findings can be used by policy makers to justify future work including policies for newborn screening in Guyana.

Limitations

- The absence of previous studies prevented comparison and interpretation of thyroid hormone levels in the neonates.
- The small sample size and single centre study prevents generalization of findings to the Guyanese population.
- Difficulties were encountered with following up participants with borderline or positive results due to their geographic locations.

Introduction

Newborn screening (NBS) is a screening test done shortly after birth to detect conditions that cause severe health problems if not treated early.[1]NBS programs have been existing for over 50 years in developed countries.[2]It allows clinical providers to identify infants at high risk for those conditions, provide diagnostic testing and initiate treatment to avoid severe outcome and to prevent death.[3]Conditions included in NBS need to have a relatively high prevalence and their testing and treatment need to be simple, inexpensive and effective.[2]While the number of conditions included in NBS panels varies across jurisdictions, the most advanced laboratory settings give NBS programs the capability for screening for over 50 conditions.[3]

Centralization of testing and advanced analytical methods such as tandem mass spectrometry which has increased the sensitivity and rapidity in evaluation have contributed to the progress made with screening neonates .[4]In lower- and middle-income jurisdictions, barriers such as lack of resources and infrastructures have limited the development of NBS programs.[4]Approximately 71% of babies worldwide are born in a jurisdiction that does not have an established NBS program.[2] As such, the majority of infants born worldwide with diseases for which NBS exists do not get diagnosed and treated early. This leads to significant mortality, morbidity and economic burden of NBS-eligible diseases in jurisdictions where NBS is not developed. [2]

Guyana currently has no NBS program and has established a partnership with Newborn Screening Ontario (NSO) to initiate screening. NSO was established in 2006 and coordinates NBS for the province of Ontario, Canada. NSO screens over 140,000 newborns per year for 29 targeted diseases by collecting and analyzing dried bloodspot (DBS) samples in the first days of life. Congenital hypothyroidism (CH) and sickle cell disease (SCD) are two relatively common conditions, that are good candidates for NBS and offered by NSO. In the Guyanese population, the prevalence of CH and SCD are unknown but there is evidence that they both have a significant public health burden with

the number of paediatric patients being seen with sickle cell anaemia and hypothyroidism as outpatients and inpatients, making these two conditions eligible for a NBS pilot in Guyana.

CH is an endocrine condition that can result in growth deficiency and severe intellectual disability if left untreated.[1]It is one of the most common preventable cause of intellectual disability with an incidence in newborns between 1:2000 and 1:4000, and as high as 1:600 newborns in iodine deficient areas. [1]CH is most often permanent and caused by an abnormality in thyroid gland development or a defect in thyroid hormonogenesis. [5]In some cases, the condition is transient, in which case it can be due to the transplacental passage of maternal medication, maternal blocking antibodies, iodine deficiency or excess. [5]Treatment for CH is effective, inexpensive and easy to manage for families.[1]Infants diagnosed with CH near birth can start treatment and avoid any growth delay or intellectual disability and develop normally.[1]However, when CH patients are identified later, treatment does not suffice to eliminate developmental and growth delays resulting from CH, which underlines the importance of NBS for CH. [1]CH was added to NBS programs starting in the mid-1970s, and has shown to be effective in reducing morbidity, mortality and the economic burden of CH: the jurisdictions screening for CH have eliminated neuro-developmental impairments resulting from CH.[2]Since establishing NBS for CH, Cuba, Puerto Rico and the US Virgin Island have been screening more than 95% of their population for CH and reported a birth incidence ranging from 1: 3,600 and 1: 9,526. [2]In Brazil, the CH prevalence has been reported as 1: 2,259. [2]The CH prevalence in Guyana remains unknown.

In Guyana, SCD was found to be among the top leading causes for admission to pediatric wards. During a one-year study, it accounted for 102 of 1380 admissions.[6]SCD is a group of recessive inherited conditions that affects the formation of haemoglobin and the functioning of red blood cells. The gene mutations responsible for SCD are most common in populations of African, South Asian, Middle Eastern and Mediterranean origins.[7]SCD is one of the most common genetic conditions worldwide, with a birth incidence estimated at 24,900 cases per year. [7]

SCD is a global health public concern, a survey conducted by the World Health Organization (WHO) reported that haemoglobin disorders such as SCD are a public health concern for 71% of the 229 countries surveyed, who represent 89% of births worldwide. [7]

While the prevalence of SCD in Guyana in unknown, data is available for some Caribbean countries through NBS. Jamaica has the highest prevalence of SCD at 0.65%, followed by Grenada (0.63%), Haiti (0.58%) and Tobago (0.57%). Trait prevalence was highest in Jamaica at 14% and St. Vincent and Grenadines (15%), followed by St. Lucia (13.8%), Haiti (13.5%) and Tobago (13.2%). [8]

SCD patients experience a wide range of adverse outcomes, including, but not limited to: anaemia, stroke, ischemic organ damage, pain crises, chronic respiratory disease, recurrent infections, and death. [9]. In 2008, the United Nations (UN) recognized sickle cell anaemia, a form of SCD, as a public health concern and urged relevant parties to strengthen health systems and primary care delivery for sickle cell anaemia. [10] Since this declaration, many jurisdictions have recognized the need for SCD NBS in order to enable early identification and treatment of SCD affected infants. [10] Several studies have concluded that NBS for SCD and early treatment for SCD leads to reduced morbidity and mortality. Notably, a study conducted in the Republic of Benin found that establishment of a NBS for SCD has led to a mortality rate 10 times lower for children under 5 years of age.[11] In North America, SCD mortality for children under 5 years of age has been almost eliminated thanks to early identification and treatment from NBS.[12]

The Georgetown Public Hospital Corporation (GPHC) partnered with NSO to conduct a pilot study of NBS for CH and SCD in Guyana. Given the infrastructure challenges for establishing and delivering NBS in Guyana, partnering with an established NBS program could help overcome obstacles and help provide data that support the implementation of a NBS program, such as disease prevalence and the NBS test's positive predictive values in the Guyanese population.

Objectives

The objectives of this pilot NBS study were:

- To assess the feasibility of implementing a NBS program in Guyana for CH and HBG
- To assess the potential benefits of implementing a NBS program in Guyana for CH and HBG
- To report on screen positive rates for CH and HBG (sickle cell disease and traits) for the Guyanese population
- To report on HBG prevalence in Guyana based on NBS results and using the Hardy-Weinberg equilibrium (HWE)

Methods

Study Design

This pilot project was a prospective descriptive study at done at GPHC from June 6th, 2016 to September 22nd, 2017.

Inclusion criteria

Term, healthy babies (gestational age of 37 weeks or later) born at GPHC during the study period whose mothers provided written consent after being briefed on the study.

Exclusion criteria

Babies with sepsis, and those admitted to the neonatal intensive care unit were excluded since their mothers were potentially under undue stress and may not have consented. Additionally, the sampling of these babies may have created additional exposure to personnel who can introduce infection and further compromise their immune system. Further sick newborns can have abnormal TSH due to their illness which may not be indicative of CH and could have cause a bias in the findings.

Sample size

This was calculated at approximately 3250 neonates based on the number of babies born annually, the duration of funding, representing. Guyana has an average of 15,000 live births per year with 22% being born at GPHC. It was not feasible to include other rural hospitals in this pilot study.

Sample Collection and handling

Shortly after birth (closer to 24 hours of life), a dried bloodspot (DBS) sample was collected by trained laboratory technicians via heel prick and using a filter card. These samples were stored at the Medical Laboratory at GPHC according to standard protocols for storing DBS. DBS filter cards were air shipped (at room temperature) weekly to NSO for analysis, with a shipping turnaround time of three days.

Analysis

Samples were analysed for CH and SCD on the day of receipt by NSO, and the NBS results were sent from NSO to GPHC within approximately 14 days of samples receipt. NBS results were categorized as: screen positive (higher probability that the infant is affected by the disease and confirmatory diagnosis testing is needed), screen negative (low probability that the infant is affected by the disease and no follow up is needed), or unsatisfactory (the sample quality did not permit the NBS test). Confirmatory testing and follow-up care were planned at GPHC.

CH

The screening test for CH was done using a human thyroid-stimulating hormone (hTSH) assay. The neonatal hTSH assay is a solid phase, two site fluoroimmunometric assay based on the direct sandwich technique. Specimens containing hTSH are reacted with anti- hTSH IgG, coated on the wells of the microtitre plates, and europium-labelled anti- hTSH IgG (Eu tracer). The wash buffer elutes hTSH from the DBS. The enhancement solution dissociates the europium ions from the

labeled antibody where they form highly fluorescent chelates. The fluorescence measured in each well is proportional to the concentration of hTSH in the sample. If the initial hTSH level in a sample was found to be greater than or equal to 13 µU/mL of blood, a confirmatory hTSH measurement was made using the same technique outlined above. If the confirmatory hTSH level was found to be greater than or equal to 17 µU/mL of blood, the newborn was considered screen positive for CH. Otherwise, the patient is said to be screen-negative for CH. Mean hTSH levels between the Guyanese sample and the Ontarian population were compared using Student's T test with an alpha of 0.05.

SCD

The screening test for SCD was performed with a cation-exchange high-performance liquid chromatography (HPLC) used to separate the different variants of hemoglobin present in a DBS elutes (reconstituted DBS). Using a two dual-piston pump, the ionic strength of the buffer is increased encouraging the more strongly retained hemoglobin to elute from the column. Each hemoglobin has a characteristic retention time. The instrument quantifies the amount of hemoglobin variants present in the sample. These include adult hemoglobins (A, A2) which are the most common types in the normal population; fetal hemoglobins (F, F1) and minor hemoglobins (E, S, C). Together, the quantitative hemoglobin results (in the form of peaks in the chromatogram) are interpreted by a clinician who assigns the chromatogram a single *hemoglobin pattern* determination. The hemoglobin pattern determination is used to determine if the newborn is said to be screen negative or screen positive for SCD. This NBS tests allows for identification of carriers of SCD.

S allele frequency was calculated using the below equation (equation 1)

(1) S allele frequency = (number of carriers) + (2 x (number of screen positive) / (number of infants screened x 2)

SCD prevalence in Guyana was estimated using a population of approximately 746,955 (according to a census conducted in 2012 conducted by the Bureau of Statistics of Guyana) [13]. The Bureau of

Statistics estimated projected births be between 13,963 and 15,864 between 2000 and 2025 in Guyana was used to calculate the birth prevalence of SCD. [13]

Outcome measures

Primary outcomes were to report on screen positive rates for CH and HBG for the Guyanese population using Hardy-Weinberg equilibrium (HWE) and percent positivity rates. The HWE is commonly used to estimate the frequency of a phenotype in a population, for example a recessive condition like SCD, because sample sizes in cross-sectional studies are usually too small to allow for a precise estimate. [14,15]. For CH, mean hTSH levels and mean difference between the Guyanese sample and the Ontarian population were compared using Student's T test with an alpha of 0.05.

Secondary outcome was to assess the feasibility of CH and SCD NBS as a public health program in Guyana using *Wilson-Jungner criteria* as the outcome measure.

Table 1: Wilson-Jungner principles [16]

Principle	Principle met?
1. The condition sought should be an important health problem.	Yes
2. There should be an accepted treatment for patients with recognized diseases	Yes
3. Facilities for diagnosis and treatment should be available	Available but was not assessed in this pilot. GPHC can diagnose and treat SCD and CH.
4. There should be a recognizable latent or early symptomatic phase	Yes
5. There should be a suitable test or examination	Yes
6. The test should be acceptable to the population	Yes
7. The natural history of the condition, including development from latent to declared disease, should be understood	Yes
8. There should be an agreed policy on whom to treat as patients	Draft document awaiting final approval by Ministry of Public Health, Guyana
9. The cost of case-finding (including diagnosis and treatment of patients diagnosed should be economically	Yes

balanced in relation to possible expenditure on medical care as a whole	
10. Case-finding should be a continuing process and not a "once and for all" project	Yes–policy brief for implementation of NBS was presented to the Pan American Heath Organization (PAHO) Guyana office in 2020 for implementation of routine NBS. No further work in on this due to COVID-pandemic.

Research Ethics

This study was reviewed and approved by the Guyana Ministry of Public Health Institutional Review Board (IRB #225) and by the Children's Hospital of Eastern Ontario Research Ethics Board (REB Protocol No: 17/210X).

Patient and Public Involvement

Patients were recruited as the sample population. Newborn babies were tested after mothers were given a written document and a brief discussion of the purpose of the research, the risks and benefits of being tested and the potential complications from a heel prick DBS sample collection. A written consent form was provided to mothers for signature. Some mothers requested that the fathers' consent as well. Only babies whose parents consented were included in the study. Data was coded with a unique identifier.

Results

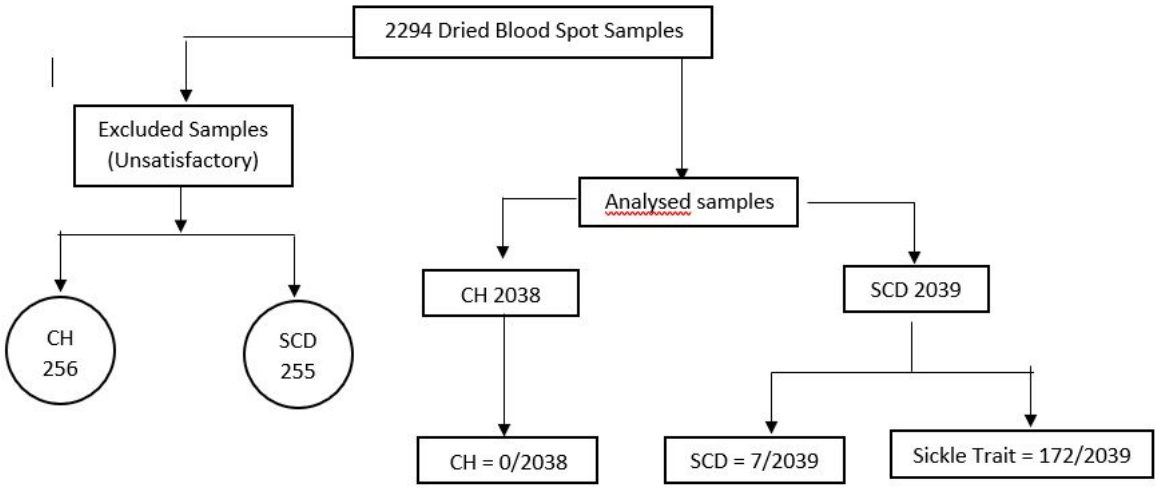

Figure 1: Flow diagram of sample collection and results

Pilot experience and feasibility

The pilot was conducted from June 6th, 2016 to September 22nd, 2017. GPHC recruited 2,294 mothers/infants. There were no reported harms to babies who were tested. All samples were successfully shipped to NSO. Confirmation of diagnosis of the screen positive neonates was not obtained since telephone numbers provided were unreachable and mothers failed to bring in their child, so this report is solely on the screen positive rate for CH and SCD, and on the estimated prevalence of SCD assuming HWE. Of note, one mother followed up and her child was confirmed as SC.

CH

Of the 2,294 received samples, 256 were excluded of CH NBS testing as they were determined unsatisfactory for testing. The most common reason for exclusion was a insufficient blood quantity of the sample (n=511). The screen positive rate for CH in our sample was 0.0% (0/2,038 infants). Mean TSH levels in Guyanese samples (1.7 µIU/mL blood) was significantly different ($p < 0.05$) than in the Ontario population (4.3 µIU/mL blood).

SCD

Of the 2,294 received samples, 255 were excluded of SCD NBS testing as they were determined unsatisfactory for testing. The most common reason for exclusion was insufficient blood quantity of the sample (n=511). The screen positive rate for SCD in our sample was 0.3% (7/2,039 patients), and the carrier rate was 8.41% (172/2,039 patients). As we were unable to obtain follow-up diagnostic data for all of the screen positive infants, the true positive rate and the positive predictive value of this SCD NBS test in the Guyanese population could not be ascertained. However, as this NBS tests identifies SCD-affected infants as well as carriers, the allele frequency for SCD in the study sample and estimate of SCD prevalence in the Guyanese population using the HWE, assuming sample is representative of the population in terms of allele frequency could be deduced. Table 1 presents the genotype and S allele frequencies in the study sample, the S allele frequency being represented by equation 1 in methods section:

Table 2: SCD genotype and S allele frequencies in the study sample

AA frequency – screen negative (%)	AS frequency – carriers (%)	SS frequency – screen positive	S allele frequency
51,35	9,07	0,34	0,049

Using the HWE, the SCD frequency is (S allele frequency) ^{2} = 0,49 ^{2} = 0.002.

Using the projected estimate birth value for 2020 of 15,126 births, it can be estimated that the birth prevalence of SCD in Guyana is approximately 30 births per year (0.2%).

CH and SCD as NBS candidate diseases in Guyana as per Wilson-Jungner criteria

To further assess the suitability of CH and SCD NBS as a public health program in Guyana, mapping of Guyana's experience was done onto the Wilson-Jungner criteria. These criteria were established in 1968 in a report for the World Health Organization[17]and have been developed as an evaluation

framework to determine the eligibility of a given condition and its test for a screening program. The Wilson-Jungner principle have been widely used as a checklist to establish NBS programs.[17]The principles acknowledge the technical characteristics of a given NBS test as well the significance of political, economic, social and health issues of an NBS test or program[16]. Table 2 presents the 10 Wilson-Jungner principles and whether each principle was met in this pilot study. Seven out of the ten principles were met in the study. Principle 3 was not assessed in this pilot but is met locally. Principles 8 and 10 were being addressed prior to the pandemic and is currently on hold due to prioritization of resources, see table 1 in methods section.

Discussion

A pilot study of NBS in Guyana was conducted in which a remote laboratory performed the screening tests. The pilot has shed light on the feasibility and potential benefits of NBS for CH and SCD in the Guyanese population. This pilot study also provided an estimate of the SCD prevalence and birth prevalence in Guyana.

There were no screen positive infants for CH in the sample population, but the researchers are familiar with cases of CH in Guyana. Small sample population of 2294 was probably responsible for zero positivity for CH as incidence ranges from 1:2000 to 1:4000. At least 4000-5000 babies will need screening for one positive sample. [1] Currently, children are tested for hypothyroidism in Guyana if there is a high index of suspicion such as developmental delay. Late diagnosis and intervention have adverse prognostic factors for these children. Further research is needed to determine the prevalence of CH in newborns and the potential causes of the mean TSH levels difference between Guyana and Ontario, and whether TSH cut-offs for CH NBS in Guyana have to be adjusted accordingly. It would notably be important to determine whether the shipment time and conditions (temperature, humidity, etc.) from Guyana to Ontario, Canada affects TSH levels in the DBS, or if mean TSH levels are different in the Guyanese population due to other factors specific to this

population. It is possible that some infants in the study sample are in fact affected by CH with TSH levels higher than the mean TSH level for the sample population. Diagnosis of CH could not have been confirmed due to lack of follow up data. Confirming diagnosis would further help determining if the TSH cut-off for CH NBS tests in Guyana should be different than the one used in Ontario by NSO. Currently, we are unable to comment on recommending CH as a priority NBS in Guyana.

The sample's screen positive rate for SCD (0.3%), as well as the estimated SCD allele frequency and birth prevalence of SCD in Guyana assuming HWE (0.2%) are lower than what has been reported in some Caribbean jurisdictions.[8] This may be due to small sample size, insufficient statistical power and insufficient external validity of the study sample, and centralized testing at GPHC without having a good representation of the rural areas in the sample population as SCD is higher in Afro-Caribbean people. To our knowledge, this is the first report of an estimated prevalence and birth prevalence of SCD in Guyana, and further research is needed to clarify these findings. The estimated birth prevalence from this study suggests that about 30 newborns per year in Guyana are affected by SCD, which indicates that Guyana may benefit from SCD NBS. Guyana recently concluded a draft document for the management of SCD and Thalassemia and there is an increase in speciality service for this population.

The eligibility of CH and SCD NBS in Guyana is further supported by our assessment of this pilot's experience using the Wilson-Jungner principles. Only three of the ten principles have not been met in this study. These three unmet principles were out of the study's scope and could be established in future work. Principle three (3), *Facilities for diagnosis and treatment should be available*, Principle eight (8), *There should be an agreed policy on whom to treat as patients*, and Principle ten (10), *Case-finding should be a continuing process and not a "once and for all" project,* could be met through some policy work in Guyana. Bibi A. Alladin has developed a policy brief for the

implementation of NBS for HGB in Guyana under the guidance of Pan American Health Organization (PAHO).

Overall, our experience suggests that NBS for CH and SCD in Guyana could be beneficial, and that it is feasible to establish a program by shipping DBS samples to a remote NBS lab for analysis while Guyana builds capacity for local testing.

Future work should focus on conducting larger pilots that would allow more diagnostic data to be collected, which in turn would allow a more accurate estimate of positive predictive values of the NBS tests and a better estimation of the mortality and morbidity reduction in Guyana if NBS is implemented. Additionally, these finding could be used to inform diagnosis and treatment guidelines for Guyanese people.

Limitations

Lack of access to testing facilities in Guyana was a major hinderance to the start of this project until NSO became involved. Only children born centrally were included in the study due to lack of resources and access to rural areas. Sample population may not be a true representation of the Guyanese population distribution. There were no positive cases of CH due to small sample size as CH prevalence ranges from 1:2000 to 1:9000. Larger studies with more robust methods for diagnostic follow up are needed to establish prevalence of CH in newborns in Guyana, limited funding did not permit a larger study at this time.

Sample rejection was 11%, this was due to lack of familiarity of DBS as a routine method of sample collection. Technicians were trained for DBS but skill set varied among technicians. This further reduced our sample size for analysis.

We were unable to follow up most of the screen positive cases for SCD despite many attempts as these cases were all from the rural areas and contact information was not reliable.

Contributorship statement

Bibi A. Alladin, Pheona Mohamed-Rambaran, Vijay Grey and Andrea Hunter developed the proposal. Bibi A. Alladin and Pheona Mohamed-Rambaran provided resources and supervision for sample collection, shipment and follow up of results locally, including reporting and patient contact. Bibi A. Alladin secured funding, completed the final manuscript and article submission. Pranesh Chakraborty, Matthew Henderson, Jennifer Milburn, Laure A. Tessier contributed to the methodology, provided resources for analyzing samples, interpretation of the results and assisted with compilation of the manuscript.

Competing interests

None

Funding

Grant number: Not applicable

This project was funded by Guyana Bank for Trading and Industry (GBTI) through a public-private partnership. Funds were used to purchase supplies for sample collection and for testing. Georgetown Public Hospital Corporation (GPHC) paid for the shipment of samples to NSO weekly and provided human resources for sample collection.

Data sharing statement

Extra data is available and can be accessed by emailing pheonar@yahoo.com.

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Dr. Jennifer McKnight provided information on NBS in the Caribbean and has volunteered to assist with implementation of NBS in Guyana.

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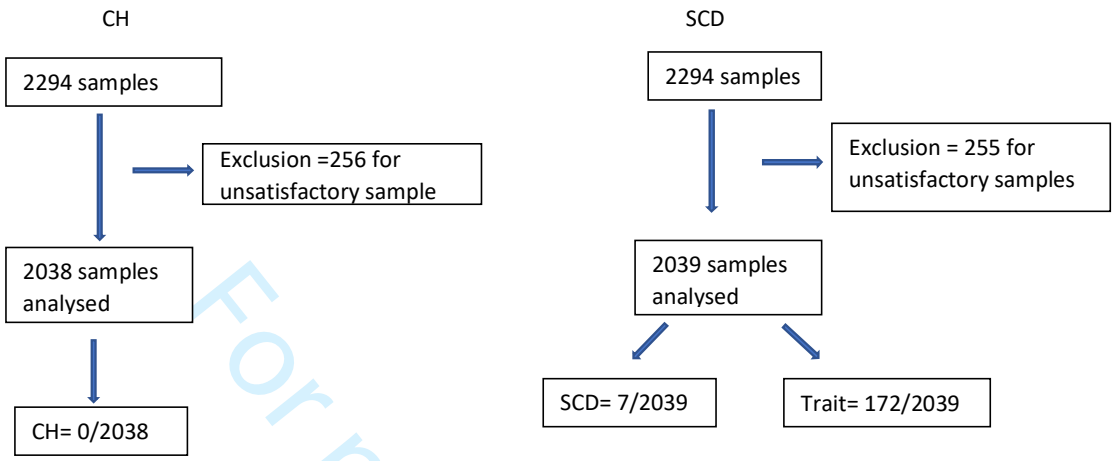
16 Jungner L, Jungner I, Engvall M, Döbeln U von: Gunnar Jungner and the Principles and Practice of Screening for Disease. Int J Neonatal Screen 2017;3:23.

17 Raffle AE, Gray JAM (John AM: Screening : evidence and practice. Oxford University Press, 2007.

Flow diagram summarizing sample collection and analysis for CH and SCD



CONSORT 2010 checklist of information to include when reporting a pilot or feasibility trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a pilot or feasibility randomised trial in the title	1
	1b	Structured summary of pilot trial design, methods, results, and conclusions (for specific guidance see CONSORT abstract extension for pilot trials)	1
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale for future definitive trial, and reasons for randomised pilot trial	2-5
	2b	Specific objectives or research questions for pilot trial	5
Methods			
Trial design	3a	Description of pilot trial design (such as parallel, factorial) including allocation ratio	
	3b	Important changes to methods after pilot trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	5-6
	4b	Settings and locations where the data were collected	5-6
	4c	How participants were identified and consented	6,9,10
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	
Outcomes	6a	Completely defined prespecified assessments or measurements to address each pilot trial objective specified in 2b, including how and when they were assessed	8
	6b	Any changes to pilot trial assessments or measurements after the pilot trial commenced, with reasons	
	6c	If applicable, prespecified criteria used to judge whether, or how, to proceed with future definitive trial	
Sample size	7a	Rationale for numbers in the pilot trial	
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	
	8b	Type of randomisation(s); details of any restriction (such as blocking and block size)	
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	

Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12	Methods used to address each pilot trial objective whether qualitative or quantitative	6-8
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were approached and/or assessed for eligibility, randomly assigned, received intended treatment, and were assessed for each objective	10
	13b	For each group, losses and exclusions after randomisation, together with reasons	
Recruitment	14a	Dates defining the periods of recruitment and follow-up	
	14b	Why the pilot trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	
Numbers analysed	16	For each objective, number of participants (denominator) included in each analysis. If relevant, these numbers should be by randomised group	10
Outcomes and estimation	17	For each objective, results including expressions of uncertainty (such as 95% confidence interval) for any estimates. If relevant, these results should be by randomised group	10-12
Ancillary analyses	18	Results of any other analyses performed that could be used to inform the future definitive trial	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	10
	19a	If relevant, other important unintended consequences	
Discussion			
Limitations	20	Pilot trial limitations, addressing sources of potential bias and remaining uncertainty about feasibility	14
Generalisability	21	Generalisability (applicability) of pilot trial methods and findings to future definitive trial and other studies	13-14
Interpretation	22	Interpretation consistent with pilot trial objectives and findings, balancing potential benefits and harms, and considering other relevant evidence	12-14
	22a	Implications for progression from pilot to future definitive trial, including any proposed amendments	
Other information			
Registration	23	Registration number for pilot trial and name of trial registry	
Protocol	24	Where the pilot trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	15
	26	Ethical approval or approval by research review committee, confirmed with reference number	9

Citation: Eldridge SM, Chan CL, Campbell MJ, Bond CM, Hopewell S, Thabane L, et al. CONSORT 2010 statement: extension to randomised pilot and feasibility trials. BMJ. 2016;355.
*We strongly recommend reading this statement in conjunction with the CONSORT 2010, extension to randomised pilot and feasibility trials, Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

BMJ Open

A pilot project to determine the feasibility of implementing newborn screening for sickle cell anemia and congenital hypothyroidism in Guyana

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A pilot project to determine the feasibility of implementing newborn screening for sickle cell anemia and congenital hypothyroidism in Guyana

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Abstract

Introduction: Newborn screening (NBS) is a test done shortly after birth to detect conditions that cause severe health problems if not treated early. An estimated 71% of babies worldwide are born in jurisdictions that do not have an established NBS program. Guyana currently has no NBS program and has established a partnership with Newborn Screening Ontario (NSO) to initiate screening. Objectives: To assess the feasibility of implementing a NBS program in Guyana for congenital hypothyroidism (CH) and hemoglobinopathies (HBG) and to report on screen positive rates and prevalence (Hardy-Weinberg equilibrium (HWE) for CH and HBG.

Methods: Term, healthy Guyanese infants were evaluated (with consent) using heel prick dried blood spots (DBS) shortly after birth (closer to 24 hours of life). DBS samples were analysed at NSO. Screening test for CH was done using a human thyroid-stimulating hormone (hTSH) assay. Mean hTSH levels between the Guyanese sample and the Ontarian population were compared using Student's T test with an alpha of 0.05. Screening test for SCAHBG was performed with a cation-exchange high-performance liquid chromatography (HPLC).

Results: The pilot was conducted from June 6th, 2016, to September 22nd, 2017. GPHC recruited 2,294 mothers/infants. Screen positive rate for CH in our sample was 0.0% (0/2,038 infants). Mean TSH levels in Guyanese samples (1.7 µU/mL blood) was noticed to be significantly different than in the Ontarian population (4.3 µU/mL blood) ($p < 0.05$). Screen positive rate for Sickle Cell Anaemia (SCA) in our sample was 0.3% (7/2,039 patients), and the carrier rate was 8.41% (172/2,039patients). Using the HWE, the SCA frequency (S allele frequency) $^2 = 0.049$ $^2 = 0.002$.

Conclusion: NBS for CH and SCA in Guyana could be beneficial. Future work should focus on conducting larger pilots which could be used to inform diagnosis and treatment guidelines for Guyanese people.

Article Summary

Strengths and limitations of this study

- This first-time study on newborn screening in Guyana provides baseline data on sickle cell anemia and thyroid hormone levels.
- The findings can be used by policy makers to justify future work, including policies for newborn screening in Guyana.

Limitations

- The absence of previous studies prevented comparison and interpretation of thyroid hormone levels in the neonates.
- The small sample size and single centre study prevents generalization of findings to the Guyanese population.
- Difficulties were encountered with following up participants with borderline or positive results due to their geographic locations.

Introduction

Newborn screening (NBS) is a screening test done shortly after birth to detect conditions that cause severe health problems if not treated early.[1]NBS programs have been existing for over 50 years in developed countries.[2]It allows clinical providers to identify infants at high risk for those conditions, provide diagnostic testing and initiate treatment to avoid severe outcome and to prevent death.[3]Conditions included in NBS need to have a relatively high prevalence and their testing and treatment need to be simple, inexpensive and effective.[2]While the number of conditions included in NBS panels varies across jurisdictions, the most advanced laboratory settings give NBS programs the capability for screening for over 50 conditions.[3]

Centralization of testing and advanced analytical methods such as tandem mass spectrometry which has increased the sensitivity and rapidity in evaluation have contributed to the progress made with screening neonates .[4]In lower- and middle-income jurisdictions, barriers such as lack of resources and infrastructures have limited the development of NBS programs.[4]Approximately 71% of babies worldwide are born in a jurisdiction that does not have an established NBS program.[2] As such, the majority of infants born worldwide with diseases for which NBS exists do not get diagnosed and treated early. This leads to significant mortality, morbidity and economic burden of NBS-eligible diseases in jurisdictions where NBS is not developed. [2]

Guyana currently has no NBS program and has established a partnership with Newborn Screening Ontario (NSO) to initiate screening. NSO was established in 2006 and coordinates NBS for the province of Ontario, Canada. NSO screens over 140,000 newborns per year for twenty-nine targeted diseases by collecting and analyzing dried bloodspot (DBS) samples in the first days of life. Congenital hypothyroidism (CH) and sickle cell anaemia (SCA) are two relatively common conditions, which are good candidates for NBS and offered by NSO. In the Guyanese population, the prevalence of CH and SCA are unknown but there is evidence that they both have a significant public health burden based on the number of paediatric patients requiring care with sickle cell anaemia and

hypothyroidism as outpatients and inpatients, making these two conditions eligible for a NBS pilot in Guyana.

CH is an endocrine condition that can result in growth deficiency and severe intellectual disability if left untreated.[1]It is one of the most common preventable cause of intellectual disability with an incidence in newborns between 1:2000 and 1:4000, and as high as 1:600 newborns in iodine deficient areas. [1]CH is most often permanent and is caused by an abnormality in thyroid gland development or a defect in thyroid hormonogenesis. [5]In some cases, the condition is transient, in which case it can be due to the transplacental passage of maternal medications, maternal blocking antibodies, iodine deficiency or excess. [5]Treatment for CH is effective, inexpensive and easy to manage for families.[1]Infants diagnosed with CH near birth can start treatment and avoid any growth delay or intellectual disability and develop normally.[1]However, when CH patients are identified later, treatment does not suffice to eliminate developmental and growth delays resulting from CH, which underlines the importance of NBS for CH. [1]CH was added to NBS programs starting in the mid-1970s, and has shown to be effective in reducing morbidity, mortality and the economic burden of CH: the jurisdictions screening for CH have eliminated neuro-developmental impairments resulting from CH.[2]Since establishing NBS for CH, Cuba, Puerto Rico and the US Virgin Island have been screening more than 95% of their population for CH and reported a birth incidence ranging from 1: 3,600 and 1: 9,526. [2]In Brazil, the CH prevalence has been reported as 1: 2,259. [2]The CH prevalence in Guyana remains unknown.

In Guyana, SCA is among the leading causes for admission to pediatric wards. During a one-year study, it accounted for 102 of 1380 admissions.[6]SCA is a group of autosomal recessive inherited conditions that affects the formation of haemoglobin and the functioning of red blood cells. The gene mutations responsible for SCA are most common in populations of African, South Asian, Middle Eastern and Mediterranean origins.[7]SCA is one of the most common genetic conditions worldwide. The global birth prevalence of homozygous sickle cell anaemia is estimated to be 111.91 per 100,000

live births with disparities by region. Africa has a birth prevalence of 1125.49 per 100,000 compared with 43.12 per 100,000 in Europe.[8]

SCA is a global health public concern, a survey conducted by the World Health Organization (WHO) reported that haemoglobin disorders such as SCA are a public health concern for 71% of the 229 countries surveyed, who represent 89% of births worldwide. [9]

While the prevalence of SCA in Guyana in unknown, data is available for some Caribbean countries through NBS. Jamaica has the highest prevalence of SCA at 0.65%, followed by Grenada (0.63%), Haiti (0.58%) and Tobago (0.57%). Trait prevalence was highest in Jamaica at 14% and St. Vincent and Grenadines (15%), followed by St. Lucia (13.8%), Haiti (13.5%) and Tobago (13.2%). [10]

Sickle Cell Disease (SCD) patients experience a wide range of adverse outcomes, including, but not limited to: anaemia, stroke, ischemic organ damage, pain crises, chronic respiratory disease, recurrent infections, and death. [11]. In 2008, the United Nations (UN) recognized SCA, a form of SCD , as a public health concern and urged relevant parties to strengthen health systems and primary care delivery for SCA. [12] Since this declaration, many jurisdictions have recognized the need for SCA NBS in order to enable early identification and treatment of SCA affected infants. [12] Several studies have concluded that NBS for SCA and early treatment for SCA leads to reduced morbidity and mortality. Notably, Rahimy et al found that establishment of a NBS for SCA has led to a mortality rate 10 times lower for children under 5 years of age in the Republic of Benin.[13] In North America, SCA mortality for children under 5 years of age has been almost eliminated thanks to early identification and treatment from NBS.[14]

The Georgetown Public Hospital Corporation (GPHC) partnered with NSO to conduct a pilot study of NBS for CH and SCA in Guyana. Given the infrastructure challenges for establishing and delivering NBS in Guyana, partnering with an established NBS program could help overcome obstacles and help provide data that support the implementation of a NBS program, such as disease prevalence and the NBS test's positive predictive values in the Guyanese population.

Objectives

The objectives of this pilot NBS study were:

- To assess the feasibility of implementing a NBS program in Guyana for CH and HBG
- To assess the potential benefits of implementing a NBS program in Guyana for CH and HBG
- To report on screen positive rates for CH and HBG (sickle cell disease and traits) for the Guyanese population
- To report on HBG prevalence in Guyana based on NBS results and using the Hardy-Weinberg equilibrium (HWE)

Methods

Study Design

This pilot project was a prospective descriptive study at done at GPHC from June 6th, 2016 to September 22nd, 2017.

Inclusion criteria

Term, healthy babies (gestational age of 37 weeks or later) born at GPHC during the study period whose mothers provided written consent after being briefed on the study.

Exclusion criteria

Babies with sepsis, and those admitted to the neonatal intensive care unit were excluded since their mothers were potentially under undue stress and may not have consented. Additionally, the sampling of these babies may have created additional exposure to personnel who can introduce infection and further compromise their immune system. Further, sick newborns can have abnormal

TSH due to their illness which may not be indicative of CH and could have cause a bias in the findings.

Sample size

The sample size was calculated to be 3250 neonates based on the number of babies born annually, the duration of funding. Guyana has an average of 15,000 live births per year with 22% being born at GPHC. It was not feasible to include rural hospitals in this pilot study.

Sample Collection and handling

Newborn babies were tested after mothers were given a written document and a brief discussion of the purpose of the research, the risks and benefits of being tested and the potential complications from a heel prick DBS sample collection. A written consent form was provided to mothers for signature. Some mothers requested that the fathers' consent as well. Only babies whose parents consented were included in the study. Data was coded with a unique identifier.

A dried bloodspot (DBS) sample was collected by trained laboratory technicians via heel prick and using a filter card shortly after birth (closer to 24 hours of life). These samples were stored at the Medical Laboratory at GPHC according to standard protocols for storing DBS. DBS filter cards were air shipped (at room temperature) weekly to NSO for analysis, with a shipping turnaround time of three days.

Analysis

Samples were analysed for CH and SCA on the day of receipt by NSO, and the NBS results were sent from NSO to GPHC within 14 days of samples receipt. NBS results were categorized as: screen positive (higher probability that the infant is affected by the disease and confirmatory diagnosis testing is needed), screen negative (low probability that the infant is affected by the disease and no

follow up is needed), or unsatisfactory (the sample quality did not permit the NBS test). Confirmatory testing and follow-up care were planned at GPHC.

CH

The screening test for CH was done using a human thyroid-stimulating hormone (hTSH) assay. The neonatal hTSH assay is a solid phase, two-site fluoroimmunometric assay based on the direct sandwich technique. Specimens containing hTSH are reacted with anti-hTSH IgG, coated on the wells of the microtitre plates, and europium-labelled anti- hTSH IgG (Eu tracer). The wash buffer elutes hTSH from the DBS. The enhancement solution dissociates the europium ions from the labeled antibody where they form highly fluorescent chelates. The fluorescence measured in each well is proportional to the concentration of hTSH in the sample. If the initial hTSH level in a sample was found to be greater than or equal to 13 µU/mL of blood, a confirmatory hTSH measurement was made using the same technique outlined above. If the confirmatory hTSH level was found to be greater than or equal to 17 µU/mL of blood, the newborn was considered screen positive for CH. Otherwise, the patient categorized to be screen-negative for CH. Mean hTSH levels between the Guyanese sample and the Ontarian population were compared using Student's T test with an alpha of 0.05.

SCA

The screening test for SCA was performed with a cation-exchange high-performance liquid chromatography (HPLC) used to separate the different variants of hemoglobin present in a DBS elutes (reconstituted DBS). Using a two dual-piston pump, the ionic strength of the buffer is increased encouraging the more strongly retained hemoglobin to elute from the column. Each hemoglobin has a characteristic retention time. The instrument quantifies the amount of hemoglobin variants present in the sample. These include adult hemoglobins (A, A2) which are the most common types in the normal population; fetal hemoglobins (F, F1) and minor hemoglobins (E,

S, C). Together, the quantitative hemoglobin results (in the form of peaks in the chromatogram) are interpreted by a clinician who assigns the chromatogram a single *hemoglobin pattern* determination. The hemoglobin pattern determination was used to determine if the newborn was screen negative or screen positive for SCA. This NBS tests allows for identification of carriers of SCA.

S allele frequency was calculated using the below equation (equation 1)

(1) S allele frequency = (number of carriers) + (2 x (number of screen positive) / (number of infants screened x 2)

SCA prevalence in Guyana was estimated using a population of approximately 746,955 (according to a census conducted in 2012 conducted by the Bureau of Statistics of Guyana) [13]. The Bureau of Statistics estimated projected births be between 13,963 and 15,864 between 2000 and 2025 in Guyana was used to calculate the birth prevalence of SCA. [15]

Outcome measures

Primary outcomes were to report on screen positive rates for CH and HBG for the Guyanese population using Hardy-Weinberg equilibrium (HWE) and percent positivity rates. The HWE is commonly used to estimate the frequency of a phenotype in a population, for example a recessive condition like SCA, because sample sizes in cross-sectional studies are usually too small to allow for a precise estimate. [16,9]. For CH, mean hTSH levels and mean difference between the Guyanese sample and the Ontarian population were compared using Student's T test with an alpha of 0.05.

Secondary outcome was to assess the feasibility of CH and SCA NBS as a public health program in Guyana using *Wilson-Jungner criteria* as the outcome measure.

Table 1: Wilson-Jungner principles [17]

Principle	Principle met?
1. The condition sought should be an important health problem.	Yes

2. There should be an accepted treatment for patients with recognized diseases	Yes
3. Facilities for diagnosis and treatment should be available	Available but was not assessed in this pilot. GPHC can diagnose and treat SCA and CH.
4. There should be a recognizable latent or early symptomatic phase	Yes
5. There should be a suitable test or examination	Yes
6. The test should be acceptable to the population	Yes
7. The natural history of the condition, including development from latent to declared disease, should be understood	Yes
8. There should be an agreed policy on whom to treat as patients	Draft document awaiting final approval by Ministry of Public Health, Guyana
9. The cost of case-finding (including diagnosis and treatment of patients diagnosed should be economically balanced in relation to possible expenditure on medical care as a whole	Yes
10. Case-finding should be a continuing process and not a "once and for all" project	Yes–policy brief for implementation of NBS was presented to the Pan American Heath Organization (PAHO) Guyana office in 2020 for implementation of routine NBS. No further work in on this due to COVID-pandemic.

Research Ethics

This study was reviewed and approved by the Guyana Ministry of Public Health Institutional Review Board (IRB #225) and by the Children's Hospital of Eastern Ontario Research Ethics Board (REB Protocol No: 17/210X).

Patient and Public Involvement

Patients were recruited as the sample population after sensitization of their mothers.

Results

Pilot experience and feasibility

The pilot was conducted from June 6th, 2016, to September 22nd, 2017. GPHC recruited 2,294 mothers/infants. There were no reported harms to babies who were assessed. All samples were successfully shipped to NSO. Confirmation of diagnosis of the screen positive neonates was not obtained since telephone numbers provided were unreachable and mothers failed to bring in their child, so this report is solely on the screen positive rate for CH and SCA, and on the estimated prevalence of SCA assuming HWE. Of note, one mother followed up and her child was confirmed as SCA.

CH

Of the 2,294 received samples, 256 were excluded of CH NBS testing as they were determined unsatisfactory for testing (Figure 1). The most common reason for exclusion was an insufficient blood quantity of the sample. The screen positive rate for CH in our sample was 0.0% (0/2,038 infants). Mean TSH levels in Guyanese samples (1.7 µIU/mL blood) was significantly different ($p < 0.05$) than in the Ontarian population (4.3 µIU/mL blood).

SCA

Of the 2,294 received samples, 255 were excluded of SCA NBS testing as they were determined unsatisfactory for testing (Figure 1). The most common reason for exclusion was insufficient blood quantity of the sample. The screen positive rate for SCA in our sample was 0.3% (7/2,039 patients), and the carrier rate was 8.41% (172/2,039 patients). As we were unable to obtain follow-up diagnostic data for all the screen positive infants, the true positive rate and the positive predictive value of this SCA NBS test in the Guyanese population could not be ascertained. However, as this NBS test identifies SCA-affected infants as well as carriers, the allele frequency for SCA in the study sample and estimate of SCA prevalence in the Guyanese population using the HWE, assuming sample is representative of the population in terms of allele frequency, could be deduced. Table 1

presents the genotype and S allele frequencies in the study sample; the S allele frequency being represented by equation 1 in methods section:

Table 2: SCA genotype and S allele frequencies in the study sample

AA frequency – screen negative (%)	AS frequency – carriers (%)	SS frequency – screen positive	S allele frequency
51.35	9.07	0.34	0.049

Using the HWE, the SCA frequency is (S allele frequency) ^{2} = 0.049 ^{2} = 0.002.

Using the projected estimate birth value for 2020 of 15,126 births, it can be estimated that the birth prevalence of SCA in Guyana is 30 births per year (0.2%).

CH and SCA as NBS candidate diseases in Guyana as per Wilson-Jungner criteria

To further assess the suitability of CH and SCA NBS as a public health program in Guyana, mapping of Guyana's experience was done onto the Wilson-Jungner criteria. These criteria were established in 1968 in a report for the World Health Organization[18]and have been developed as an evaluation framework to determine the eligibility of a given condition and its test for a screening program. The Wilson-Jungner principle have been widely used as a checklist to establish NBS programs.[17]The principles acknowledge the technical characteristics of a given NBS test as well the significance of political, economic, social and health issues of an NBS test or program[17]. Table 2 presents the 10 Wilson-Jungner principles and whether each principle was met in this pilot study. Seven out of the ten principles were met in the study. Principle 3 was not assessed in this pilot but is met locally. Principles 8 and 10 were being addressed prior to the pandemic and is currently on hold due to prioritization of resources, see table 1 in methods section.

Discussion

A pilot study of NBS in Guyana was conducted in which a remote laboratory performed the screening tests. The pilot has shed light on the feasibility and potential benefits of NBS for CH and SCA in the Guyanese population. This pilot study also provided an estimate of the SCA prevalence and birth prevalence in Guyana.

There were no screen positive infants for CH in the sample population, but the researchers are familiar with cases of CH in Guyana. Small sample population of 2294 was responsible for zero positivity for CH as incidence ranges from 1:2000 to 1:4000. At least 4000-5000 babies will need screening for one positive sample. [1] Currently, children are evaluated for hypothyroidism in Guyana if there is a high index of suspicion such as developmental delay. Late diagnosis and intervention have adverse prognostic factors for these children. Further research is needed to determine the prevalence of CH in newborns and the potential causes of the mean TSH levels difference between Guyana and Ontario, and whether TSH cut-offs for CH NBS in Guyana must be adjusted accordingly. It would notably be important to determine whether the shipment time and conditions (temperature, humidity) from Guyana to Ontario, Canada affect TSH levels in the DBS, or if mean TSH levels are different in the Guyanese population due to other factors specific to this population. It is possible that some infants in the study sample are in fact affected by CH with TSH levels higher than the mean TSH level for the sample population. Diagnosis of CH could not have been confirmed due to lack of follow up data. Confirming diagnosis would further help determine if the TSH cut-off for CH NBS tests in Guyana should be different than the one used in Ontario by NSO. Currently, we are unable to comment on recommending CH as a priority NBS in Guyana.

The sample's screen positive rate for SCA (0.3%), as well as the estimated SCA allele frequency and birth prevalence of SCA in Guyana assuming HWE (0.2%) are lower than what has been reported in some Caribbean jurisdictions.[10] This may be due to small sample size, insufficient statistical power and insufficient external validity of the study sample, and centralized testing at GPHC without having

a good representation of the rural areas in the sample population as SCA is higher in Afro-Caribbean people. To our knowledge, this is the first report of an estimated prevalence and birth prevalence of SCA in Guyana, and further research is needed to clarify these findings. The estimated birth prevalence from this study suggests that about thirty newborns per year in Guyana are affected by SCA, which indicates that Guyana may benefit from SCA NBS. Guyana recently concluded a draft document for the management of SCA and Thalassemia and there is an increase in speciality service for this population. Guyana has a significant East Indian ancestry with genetic susceptibility to Thalassemia, this population may benefit from HGB screening that may identify beta Thalassemia.

The eligibility of CH and SCA NBS in Guyana is further supported by our assessment of this pilot's experience using the Wilson-Jungner principles. Only three of the ten principles have not been met in this study. These three unmet principles were out of the study's scope and could be established in future work. Principle three (3), *Facilities for diagnosis and treatment should be available*, Principle eight (8), *There should be an agreed policy on whom to treat as patients*, and Principle ten (10), *Case-finding should be a continuing process and not a "once and for all" project,* could be met through some policy work in Guyana. Bibi A. Alladin has developed a policy brief for the implementation of NBS for HGB in Guyana under the guidance of Pan American Health Organization (PAHO).

Overall, our experience suggests that NBS for CH and SCA in Guyana could be beneficial, and that it is feasible to establish a program by shipping DBS samples to a remote NBS lab for analysis while Guyana builds capacity for local testing. Over the last three years, Guyana has dispatched more doctors to the rural areas as the medical capacity continues to grow, this may help to mitigate the challenges with following up of screen positive samples from rural areas through the network of primary health care.

Future work should focus on conducting larger pilots that would allow more diagnostic data to be collected, which in turn would allow a more accurate estimate of positive predictive values of the NBS tests and a better estimation of the mortality and morbidity reduction in Guyana if NBS is implemented. Additionally, these finding could be used to inform diagnosis and treatment guidelines for Guyanese people.

Limitations

Lack of access to testing facilities in Guyana was a major hinderance to the start of this project until NSO became involved. Only children born centrally were included in the study due to lack of resources and access to rural areas. Sample population may not be a true representation of the Guyanese population distribution. There were no positive cases of CH due to small sample size as CH prevalence ranges from 1:2000 to 1:9000. Larger studies with more robust methods for diagnostic follow up are needed to establish prevalence of CH in newborns in Guyana, limited funding did not permit a larger study at this time.

Sample rejection was 11%, this was due to lack of familiarity of DBS as a routine method of sample collection. Technicians were trained for DBS, but skill set varied among technicians. This further reduced our sample size for analysis.

We were unable to follow up most of the screen positive cases for SCA despite many attempts as these cases were all from the rural areas and contact information was not reliable.

Contributorship statement

Bibi A. Alladin, Pheona Mohamed-Rambaran, Vijay Grey and Andrea Hunter developed the proposal. Bibi A. Alladin and Pheona Mohamed-Rambaran provided resources and supervision for sample collection, shipment and follow up of results locally, including reporting and patient contact and completed the final manuscript

Pranesh Chakraborty, Matthew Henderson, Jennifer Milburn, Laure A. Tessier contributed to the methodology, provided resources for analyzing samples, interpretation of the results and assisted with compilation of the manuscript.

Bibi A. Alladin secured funding.

Competing interests

None

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Grant number: Not applicable

This project was funded by Guyana Bank for Trading and Industry (GBTI) through a public-private partnership. Funds were used to purchase supplies for sample collection and for testing. Georgetown Public Hospital Corporation (GPHC) paid for the shipment of samples to NSO weekly and provided human resources for sample collection.

Data sharing statement

Extra data is available and can be accessed by emailing Pheona Mohamed-Rambaran at pheonar@yahoo.com.

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Dr. Jennifer McKnight provided information on NBS in the Caribbean and has volunteered to assist with implementation of NBS in Guyana.

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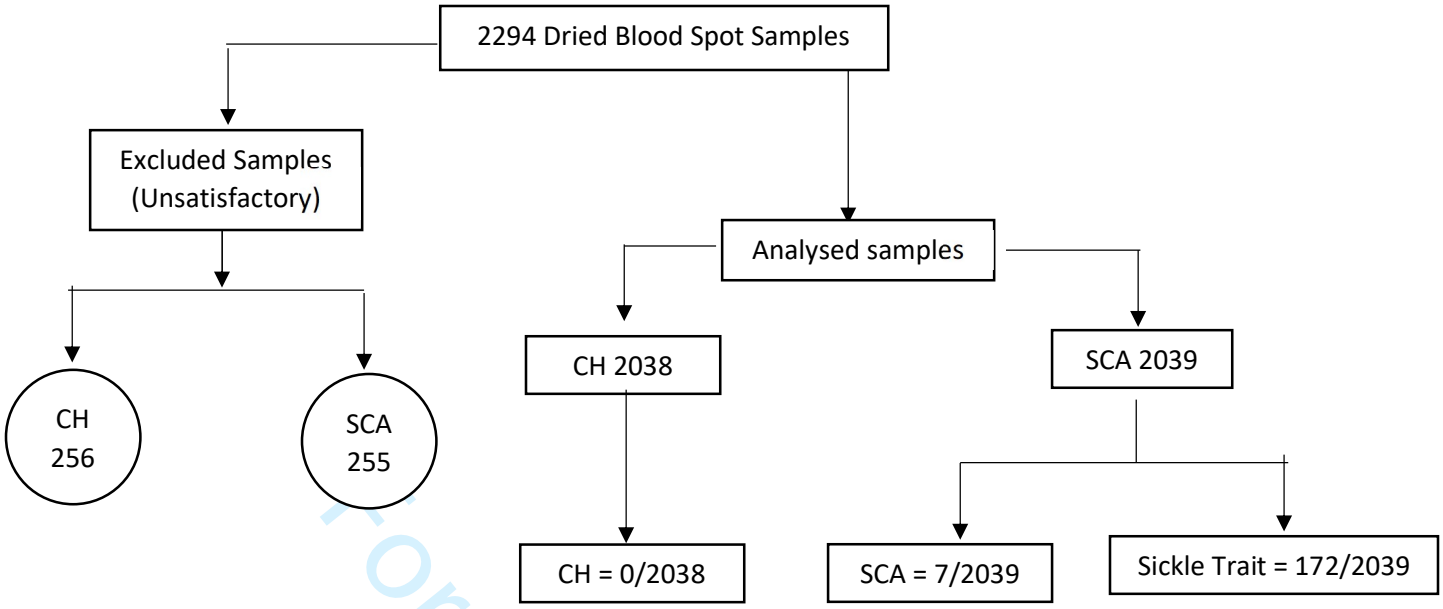

Figure 1: Flow diagram of sample collection and results

CONSORT 2010 checklist of information to include when reporting a pilot or feasibility trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a pilot or feasibility randomised trial in the title	1
	1b	Structured summary of pilot trial design, methods, results, and conclusions (for specific guidance see CONSORT abstract extension for pilot trials)	1
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale for future definitive trial, and reasons for randomised pilot trial	2-5
	2b	Specific objectives or research questions for pilot trial	5
Methods			
Trial design	3a	Description of pilot trial design (such as parallel, factorial) including allocation ratio	
	3b	Important changes to methods after pilot trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	5-6
	4b	Settings and locations where the data were collected	5-6
	4c	How participants were identified and consented	6,9,10
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	
Outcomes	6a	Completely defined prespecified assessments or measurements to address each pilot trial objective specified in 2b, including how and when they were assessed	8
	6b	Any changes to pilot trial assessments or measurements after the pilot trial commenced, with reasons	
	6c	If applicable, prespecified criteria used to judge whether, or how, to proceed with future definitive trial	
Sample size	7a	Rationale for numbers in the pilot trial	
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	
	8b	Type of randomisation(s); details of any restriction (such as blocking and block size)	
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	

Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12	Methods used to address each pilot trial objective whether qualitative or quantitative	6-8
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were approached and/or assessed for eligibility, randomly assigned, received intended treatment, and were assessed for each objective	10
	13b	For each group, losses and exclusions after randomisation, together with reasons	
Recruitment	14a	Dates defining the periods of recruitment and follow-up	
	14b	Why the pilot trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	
Numbers analysed	16	For each objective, number of participants (denominator) included in each analysis. If relevant, these numbers should be by randomised group	10
Outcomes and estimation	17	For each objective, results including expressions of uncertainty (such as 95% confidence interval) for any estimates. If relevant, these results should be by randomised group	10-12
Ancillary analyses	18	Results of any other analyses performed that could be used to inform the future definitive trial	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	10
	19a	If relevant, other important unintended consequences	
Discussion			
Limitations	20	Pilot trial limitations, addressing sources of potential bias and remaining uncertainty about feasibility	14
Generalisability	21	Generalisability (applicability) of pilot trial methods and findings to future definitive trial and other studies	13-14
Interpretation	22	Interpretation consistent with pilot trial objectives and findings, balancing potential benefits and harms, and considering other relevant evidence	12-14
	22a	Implications for progression from pilot to future definitive trial, including any proposed amendments	
Other information			
Registration	23	Registration number for pilot trial and name of trial registry	
Protocol	24	Where the pilot trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	15
	26	Ethical approval or approval by research review committee, confirmed with reference number	9

Citation: Eldridge SM, Chan CL, Campbell MJ, Bond CM, Hopewell S, Thabane L, et al. CONSORT 2010 statement: extension to randomised pilot and feasibility trials. BMJ. 2016;355.

*We strongly recommend reading this statement in conjunction with the CONSORT 2010, extension to randomised pilot and feasibility trials, Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

BMJ Open

A cross-sectional prospective study to determine the feasibility of newborn screening for sickle cell anemia and congenital hypothyroidism in Guyana

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A cross-sectional prospective study to determine the feasibility of newborn screening for sickle cell anemia and congenital hypothyroidism in Guyana

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Abstract

Introduction: Newborn screening (NBS) is a test done shortly after birth to detect conditions that cause severe health problems if not treated early. An estimated 71% of babies worldwide are born in jurisdictions that do not have an established NBS program. Guyana currently has no NBS program and has established a partnership with Newborn Screening Ontario (NSO) to initiate screening. Objectives: To assess the feasibility of implementing a NBS program in Guyana for congenital hypothyroidism (CH) and hemoglobinopathies (HBG) and to report on screen positive rates and prevalence (Hardy-Weinberg equilibrium (HWE) for CH and HBG.

Methods: Term, healthy Guyanese infants were evaluated (with consent) using heel prick dried blood spots (DBS) shortly after birth (closer to 24 hours of life). DBS samples were analysed at NSO. Screening test for CH was done using a human thyroid-stimulating hormone (hTSH) assay. Mean hTSH levels between the Guyanese sample and the Ontarian population were compared using Student's T test with an alpha of 0.05. Screening test for SHBG was performed with a cation-exchange high-performance liquid chromatography (HPLC).

Results: The pilot was conducted from June 6th, 2016, to September 22nd, 2017. GPHC recruited 2,294 mothers/infants. Screen positive rate for CH in our sample was 0.0% (0/2,038 infants). Mean TSH levels in Guyanese samples (1.7 µU/mL blood) was noticed to be significantly different than in the Ontarian population (4.3 µU/mL blood) ($p < 0.05$). Screen positive rate for Sickle Cell Anaemia (SCA) in our sample was 0.3% (7/2,039 patients), and the carrier rate was 8.41% (172/2,039patients). Using the HWE, the SCA frequency (S allele frequency) ^{2} is $0.049^2 = 0.002$

Conclusion: NBS for CH and SCA in Guyana could be beneficial. Future work should focus on conducting larger pilots which could be used to inform diagnosis and treatment guidelines for Guyanese people.

Article Summary

Strengths and limitations of this study

- This first-time study on newborn screening in Guyana provides baseline data on sickle cell anemia and thyroid hormone levels.
- The findings can be used by policy makers to justify future work, including policies for newborn screening in Guyana.

Limitations

- The absence of previous studies prevented comparison and interpretation of thyroid hormone levels in the neonates.
- The small sample size and single centre study prevents generalization of findings to the Guyanese population.
- Difficulties were encountered with following up of participants with borderline or positive results due to their geographic locations.

Introduction

Newborn screening (NBS) is a screening test done shortly after birth to detect conditions that cause severe health problems if not treated early.[1]NBS programs have been existing for over 50 years in developed countries.[2]It allows clinical providers to identify infants at high risk for those conditions, provide diagnostic testing, and initiate treatment to avoid severe outcome and to prevent death.[3]Conditions included in NBS need to have a relatively high prevalence and their testing and treatment need to be simple, inexpensive and effective.[2]While the number of conditions included in NBS panels varies across jurisdictions, the most advanced laboratory settings give NBS programs the capability for screening for over 50 conditions.[3]

Centralization of testing and advanced analytical methods such as tandem mass spectrometry which has increased the sensitivity and rapidity in evaluation have contributed to the progress made with screening neonates .[4]In lower- and middle-income jurisdictions, barriers such as lack of resources and infrastructures have limited the development of NBS programs.[4]Approximately 71% of babies worldwide are born in a jurisdiction that does not have an established NBS program.[2] As such, the majority of infants born worldwide with diseases for which NBS exists do not get diagnosed and treated early. This leads to significant mortality, morbidity and economic burden of NBS-eligible diseases in jurisdictions where NBS is not developed. [2]

Guyana currently has no NBS program and has established a partnership with Newborn Screening Ontario (NSO) to initiate screening. NSO was established in 2006 and coordinates NBS for the province of Ontario, Canada. NSO screens over 140,000 newborns per year for twenty-nine targeted diseases by collecting and analyzing dried bloodspot (DBS) samples in the first days of life. Congenital hypothyroidism (CH) and sickle cell anaemia (SCA) are two relatively common conditions, which are good candidates for NBS and offered by NSO. In the Guyanese population, the prevalence of CH and SCA are unknown but there is evidence that they both have a significant public health

burden based on the number of paediatric patients requiring care with SCA and hypothyroidism as outpatients and inpatients, making these two conditions eligible for a NBS pilot in Guyana.

CH is an endocrine condition that can result in growth deficiency and severe intellectual disability if left untreated.[1]It is one of the most common preventable cause of intellectual disability with an incidence in newborns between 1:2000 and 1:4000, and as high as 1:600 newborns in iodine deficient areas. [1]CH is most often permanent and is caused by an abnormality in thyroid gland development or a defect in thyroid hormonogenesis. [5]In some cases, the condition is transient, in which can be due to the transplacental passage of maternal medications, maternal blocking antibodies, iodine deficiency or excess. [5]Treatment for CH is effective, inexpensive and easy to manage for families.[1]Infants diagnosed with CH near birth can start treatment and avoid any growth delay or intellectual disability and develop normally.[1]However, when CH patients are identified later, treatment does not suffice to eliminate developmental and growth delays resulting from CH, which underlines the importance of NBS for CH. [1]CH was added to NBS programs starting in the mid-1970s, and has shown to be effective in reducing morbidity, mortality and the economic burden of CH: the jurisdictions screening for CH have eliminated neuro-developmental impairments resulting from CH.[2]Since establishing NBS for CH, Cuba, Puerto Rico and the US Virgin Island have been screening more than 95% of their population for CH and reported a birth incidence ranging from 1: 3,600 and 1: 9,526. [2]In Brazil, the CH prevalence has been reported as 1: 2,259. [2]The CH prevalence in Guyana remains unknown.

In Guyana, SCA is among the leading causes for admission to pediatric wards. During a one-year study, it accounted for 102 of 1380 admissions.[6]SCA is a group of autosomal recessive inherited conditions that affects the formation of haemoglobin and the functioning of red blood cells. The gene mutations responsible for SCA are most common in populations of African, South Asian, Middle Eastern and Mediterranean origins.[7]SCA is one of the most common genetic conditions worldwide. The global birth prevalence of homozygous sickle cell anaemia is estimated to be 111.91 per 100,000

live births with disparities by region. Africa has a birth prevalence of 1125.49 per 100,000 compared with 43.12 per 100,000 in Europe.[8]

SCA is a global health public concern, a survey conducted by the World Health Organization (WHO) reported that haemoglobin disorders such as SCA are a public health concern for 71% of the 229 countries surveyed, which represent 89% of births worldwide. [9]

While the prevalence of SCA in Guyana in unknown, data is available for some Caribbean countries through NBS. Jamaica has the highest prevalence of SCA at 0.65%, followed by Grenada (0.63%), Haiti (0.58%) and Tobago (0.57%). Trait prevalence was highest in Jamaica at 14% and St. Vincent and Grenadines (15%), followed by St. Lucia (13.8%), Haiti (13.5%) and Tobago (13.2%). [10]

Sickle Cell Disease (SCD) patients experience a wide range of adverse outcomes, including, but not limited to: anaemia, stroke, ischemic organ damage, pain crises, chronic respiratory disease, recurrent infections, and death. [11]. In 2008, the United Nations (UN) recognized SCA, a form of SCD , as a public health concern and urged relevant parties to strengthen health systems and primary care delivery for SCA. [12] Since this declaration, many jurisdictions have recognized the need for SCA NBS in order to enable early identification and treatment of SCA affected infants. [12] Several studies have concluded that NBS for SCA and early treatment for SCA leads to reduced morbidity and mortality. Notably, a study conducted in the Republic of Benin found that establishment of a NBS for SCA has led to a mortality rate 10 times lower for children under 5 years of age.[13] In North America, SCA mortality for children under 5 years of age has been almost eliminated thanks to early identification and treatment from NBS.[14]

The Georgetown Public Hospital Corporation (GPHC) partnered with NSO to conduct a pilot study of NBS for CH and SCA in Guyana. Given the infrastructure challenges for establishing and delivering NBS in Guyana, partnering with an established NBS program could help overcome obstacles and help provide data that support the implementation of a NBS program, such as disease prevalence and the NBS test's positive predictive values in the Guyanese population.

Objectives

The objectives of this pilot NBS study were:

- To assess the feasibility of implementing a NBS program in Guyana for CH and HBG
- To assess the potential benefits of implementing a NBS program in Guyana for CH and HBG
- To report on screen positive rates for CH and HBG (sickle cell disease and traits) for the Guyanese population
- To report on HBG prevalence in Guyana based on NBS results and using the Hardy-Weinberg equilibrium (HWE)

Methods

Study Design

This pilot project was a prospective descriptive study at done at GPHC from June 6th, 2016 to September 22nd, 2017.

Inclusion criteria

Term, healthy babies (gestational age of 37 weeks or later) born at GPHC during the study period whose mothers provided written consent after being briefed on the study.

Exclusion criteria

Babies with sepsis, and those admitted to the neonatal intensive care unit were excluded since their mothers were potentially under undue stress and may not have consented. Additionally, the sampling of these babies may have created additional exposure to personnel who can introduce infection and further compromise their immune system. Further, sick newborns can have abnormal

TSH due to their illness which may not be indicative of CH and could have cause a bias in the findings.

Sample size

The sample size was calculated to be 3250 neonates based on the number of babies born annually, which represented the duration of funding.. Guyana has an average of 15,000 live births per year with 22% being born at GPHC. It was not feasible to include rural hospitals in this pilot study.

Sample Collection and handling

Newborn babies were tested after mothers were given a written document and a brief discussion of the purpose of the research, the risks and benefits of being tested and the potential complications from a heel prick DBS sample collection. A written consent form was provided to mothers for signature. Some mothers requested that the fathers' consent as well. Only babies whose parents consented were included in the study. Data was coded with a unique identifier.

A dried bloodspot (DBS) sample was collected by trained laboratory technicians via heel prick and using a filter card shortly after birth (closer to 24 hours of life). These samples were stored at the Medical Laboratory at GPHC according to standard protocols for storing DBS. DBS filter cards were air shipped (at room temperature) weekly to NSO for analysis, with a shipping turnaround time of three days.

Analysis

Samples were analysed for CH and SCA on the day of receipt by NSO, and the NBS results were sent from NSO to GPHC within 14 days of samples receipt. NBS results were categorized as: screen positive (higher probability that the infant is affected by the disease and confirmatory diagnosis testing is needed), screen negative (low probability that the infant is affected by the disease and no

follow up is needed), or unsatisfactory (the sample quality did not permit the NBS test). Confirmatory testing and follow-up care were planned at GPHC.

CH

The screening test for CH was done using a human thyroid-stimulating hormone (hTSH) assay. The neonatal hTSH assay is a solid phase, two-site fluoroimmunometric assay based on the direct sandwich technique. Specimens containing hTSH are reacted with anti-hTSH IgG, coated on the wells of the microtitre plates, and europium-labelled anti- hTSH IgG (Eu tracer). The wash buffer elutes hTSH from the DBS. The enhancement solution dissociates the europium ions from the labeled antibody where they form highly fluorescent chelates. The fluorescence measured in each well is proportional to the concentration of hTSH in the sample. If the initial hTSH level in a sample was found to be greater than or equal to 13 µU/mL of blood, a confirmatory hTSH measurement was made using the same technique outlined above. If the confirmatory hTSH level was found to be greater than or equal to 17 µU/mL of blood, the newborn was considered screen positive for CH. Otherwise, the patient is categrorized to be screen-negative for CH. Mean hTSH levels between the Guyanese sample and the Ontarian population were compared using Student's T test with an alpha of 0.05.

SCA

The screening test for SCA was performed with a cation-exchange high-performance liquid chromatography (HPLC) used to separate the different variants of hemoglobin present in a DBS elutes (reconstituted DBS). Using a two dual-piston pump, the ionic strength of the buffer is increased, encouraging the more strongly retained hemoglobin to elute from the column. Each hemoglobin has a characteristic retention time. The instrument quantifies the amount of hemoglobin variants present in the sample. These include adult hemoglobins (A, A2) which are the most common types in the normal population; fetal hemoglobins (F, F1) and minor hemoglobins (E,

S, C). Together, the quantitative hemoglobin results (in the form of peaks in the chromatogram) are interpreted by a clinician who assigns the chromatogram a single *hemoglobin pattern* determination. The hemoglobin pattern determination was used to determine if the newborn was screen negative or screen positive for SCA. This NBS tests allows for identification of carriers of SCA.

S allele frequency was calculated using the below equation (equation 1)

(1) S allele frequency = (number of carriers) + (2 x (number of screen positive) / (number of infants screened x 2)

SCA prevalence in Guyana was estimated using a population of approximately 746,955 (according to a census conducted in 2012 conducted by the Bureau of Statistics of Guyana). [13] The Bureau of Statistics estimated projected births be between 13,963 and 15,864 between 2000 and 2025 in Guyana was used to calculate the birth prevalence of SCA. [15]

Outcome measures

Primary outcomes were to report on screen positive rates for CH and HBG for the Guyanese population using Hardy-Weinberg equilibrium (HWE) and percent positivity rates. The HWE is commonly used to estimate the frequency of a phenotype in a population, for example a recessive condition like SCA, because sample sizes in cross-sectional studies are usually too small to allow for a precise estimate. [16,9] For CH, mean hTSH levels and mean difference between the Guyanese sample and the Ontarian population were compared using Student's T test with an alpha of 0.05.

Secondary outcome was to assess the feasibility of CH and SCA NBS as a public health program in Guyana using *Wilson-Jungner criteria* as the outcome measure (as shown in Table 1).

Table 1: Wilson-Jungner principles [17]

Principle	Principle met?
1. The condition sought should be an important health problem.	Yes

2. There should be an accepted treatment for patients with recognized diseases	Yes
3. Facilities for diagnosis and treatment should be available	Available but was not assessed in this pilot. GPHC can diagnose and treat SCA and CH.
4. There should be a recognizable latent or early symptomatic phase	Yes
5. There should be a suitable test or examination	Yes
6. The test should be acceptable to the population	Yes
7. The natural history of the condition, including development from latent to declared disease, should be understood	Yes
8. There should be an agreed policy on whom to treat as patients	Draft document awaiting final approval by Ministry of Health, Guyana
9. The cost of case-finding (including diagnosis and treatment of patients diagnosed should be economically balanced in relation to possible expenditure on medical care as a whole	Yes
10. Case-finding should be a continuing process and not a "once and for all" project	Yes–policy brief for implementation of NBS was presented to the Pan American Heath Organization (PAHO) Guyana office in 2020 for implementation of routine NBS. No further work on this due to COVID-pandemic.

Research Ethics

This study was reviewed and approved by the Guyana Ministry of Public Health Institutional Review Board (IRB #225) and by the Children's Hospital of Eastern Ontario Research Ethics Board (REB Protocol No: 17/210X).

Patient and Public Involvement

No patients or member of the public were included in the design of this study, patients were included in the data collection phase and in the reporting of results that needed follow-up. Patients were recruited as the sample population after sensitization of their mothers.

Results

Pilot experience and feasibility

The pilot was conducted from June 6th, 2016, to September 22nd, 2017. GPHC recruited 2,294 mothers/infants. There were no reported harms to babies who were assessed. All samples were successfully shipped to NSO. Confirmation of diagnosis of the screen positive neonates was not obtained since telephone numbers provided were unreachable and mothers failed to bring in their child, so this report is solely on the screen positive rate for CH and SCA, and on the estimated prevalence of SCA assuming HWE. Of note, one mother followed up and her child was confirmed as SCA.

CH

Of the 2,294 received samples, 256 were excluded from CH NBS testing as they were determined unsatisfactory for testing (Figure 1). The most common reason for exclusion was an insufficient blood quantity of the sample. The screen positive rate for CH in our sample was 0.00% (0/2,038 infants). Mean TSH levels in Guyanese samples (1.70 µIU/mL blood) was significantly different ($p < 0.05$) than in the Ontarian population (4.30 µIU/mL blood).

SCA

Of the 2,294 received samples, 255 were excluded of SCA NBS testing as they were determined unsatisfactory for testing (Figure 1). The most common reason for exclusion was insufficient blood quantity of the sample. The screen positive rate for SCA in our sample was 0.30% (7/2,039 patients), and the carrier rate was 8.41% (172/2,039 patients). As we were unable to obtain follow-up diagnostic data for all the screen positive infants, the true positive rate and the positive predictive value of this SCA NBS test in the Guyanese population could not be ascertained. However, as this NBS test identifies SCA-affected infants as well as carriers, the allele frequency for SCA in the study

sample and estimate of SCA prevalence in the Guyanese population using the HWE, assuming sample is representative of the population in terms of allele frequency, could be deduced. Table 2 presents the genotype and S allele frequencies in the study sample; the S allele frequency being represented by equation 1 in methods section:

Table 2: SCA genotype and S allele frequencies in the study sample

AA frequency – screen negative (%)	AS frequency – carriers (%)	SS frequency – screen positive	S allele frequency
51.35	9.07	0.34	0.049

Using the HWE, the SCA frequency is (S allele frequency) ^{2} = 0.049 ^{2} = 0.002.

Using the projected estimate birth value for 2020 of 15,126 births, it can be estimated that the birth prevalence of SCA in Guyana is 30 births per year (0.2%).

CH and SCA as NBS candidate diseases in Guyana as per Wilson-Jungner criteria

To further assess the suitability of CH and SCA NBS as a public health program in Guyana, mapping of Guyana's experience was done onto the Wilson-Jungner criteria from Table 1. These criteria were established in 1968 in a report for the World Health Organization[18] and have been developed as an evaluation framework to determine the eligibility of a given condition and its test for a screening program. The Wilson-Jungner principle have been widely used as a checklist to establish NBS programs.[17] The principles acknowledge the technical characteristics of a given NBS test as well the significance of political, economic, social and health issues of an NBS test or program.[17] Table 1 presents the 10 Wilson-Jungner principles and whether each principle was met in this pilot study. Seven out of the ten principles were met in the study. Principle 3 was not assessed in this pilot but is met locally. Principles 8 and 10 were being addressed prior to the pandemic and is currently on hold due to prioritization of resources.

Discussion

A pilot study of NBS in Guyana was conducted in which a remote laboratory performed the screening tests. The pilot has shed light on the feasibility and potential benefits of NBS for CH and SCA in the Guyanese population. This pilot study also provided an estimate of the SCA prevalence and birth prevalence in Guyana.

There were no screen positive infants for CH in the sample population, but the researchers are familiar with cases of CH in Guyana. Small sample population of 2294 was responsible for zero positivity for CH as incidence ranges from 1:2000 to 1:4000. At least 4000-5000 babies will need screening for one positive sample. [1] Currently, children are evaluated for hypothyroidism in Guyana if there is a high index of suspicion such as developmental delay. Late diagnosis and intervention have adverse prognostic factors for these children. Further research is needed to determine the prevalence of CH in newborns and the potential causes of the mean TSH levels difference between Guyana and Ontario, and whether TSH cut-offs for CH NBS in Guyana must be adjusted accordingly. It would notably be important to determine whether the shipment time and conditions (temperature, humidity) from Guyana to Ontario, Canada affect TSH levels in the DBS, or if mean TSH levels are different in the Guyanese population due to other factors specific to this population. It is possible that some infants in the study sample are in fact affected by CH with TSH levels higher than the mean TSH level for the sample population. Diagnosis of CH could not have been confirmed due to lack of follow up data. Confirming diagnosis would further help determine if the TSH cut-off for CH NBS tests in Guyana should be different than the one used in Ontario by NSO. Currently, we are unable to comment on recommending CH as a priority NBS in Guyana.

The sample's screen positive rate for SCA (0.3%), as well as the estimated SCA allele frequency and birth prevalence of SCA in Guyana assuming HWE (0.2%) are lower than what has been reported in some Caribbean jurisdictions.[10] This may be due to small sample size, insufficient statistical power

and insufficient external validity of the study sample, and centralized testing at GPHC without having a good representation of the rural areas in the sample population as SCA is higher in Afro-Caribbean people. To our knowledge, this is the first report of an estimated prevalence and birth prevalence of SCA in Guyana, and further research is needed to clarify these findings. The estimated birth prevalence from this study suggests that about thirty newborns per year in Guyana are affected by SCA, which indicates that Guyana may benefit from SCA NBS. Guyana recently concluded a draft document for the management of SCA and Thalassemia and there is an increase in speciality service for this population. Guyana has a significant East Indian ancestry with genetic susceptibility to Thalassemia, this population may benefit from HGB screening that may identify beta Thalassemia.

The eligibility of CH and SCA NBS in Guyana is further supported by our assessment of this pilot's experience using the Wilson-Jungner principles. Only three of the ten principles have not been met in this study. These three unmet principles were out of the study's scope and could be established in future work. Principle three (3), *Facilities for diagnosis and treatment should be available*, Principle eight (8), *There should be an agreed policy on whom to treat as patients*, and Principle ten (10), *Case-finding should be a continuing process and not a "once and for all" project,* could be met through some policy work in Guyana. Bibi A. Alladin has developed a policy brief for the implementation of NBS for HGB in Guyana under the guidance of Pan American Health Organization (PAHO).

Overall, our experience suggests that NBS for CH and SCA in Guyana could be beneficial, and that it is feasible to establish a program by shipping DBS samples to a remote NBS lab for analysis while Guyana builds capacity for local testing. Over the last three years, Guyana has dispatched more doctors to the rural areas as the medical capacity continues to grow, this may help to mitigate the challenges with following up of screen positive samples from rural areas through the network of primary health care.

Future work should focus on conducting larger pilots that would allow more diagnostic data to be collected, which in turn would allow a more accurate estimate of positive predictive values of the NBS tests and a better estimation of the mortality and morbidity reduction in Guyana if NBS is implemented. Additionally, these finding could be used to inform diagnosis and treatment guidelines for Guyanese people.

Limitations

Lack of access to testing facilities in Guyana was a major hinderance to the start of this project until NSO became involved. Only children born centrally were included in the study due to lack of resources and access to rural areas. Sample population may not be a true representation of the Guyanese population distribution. There were no positive cases of CH due to small sample size as CH prevalence ranges from 1:2000 to 1:9000. Larger studies with more robust methods for diagnostic follow up are needed to establish prevalence of CH in newborns in Guyana, limited funding did not permit a larger study at this time.

Sample rejection was 11%, this was due to lack of familiarity of DBS as a routine method of sample collection. Technicians were trained for DBS, but skill set varied among technicians. This further reduced our sample size for analysis.

We were unable to follow up most of the screen positive cases for SCA despite many attempts as these cases were all from the rural areas and contact information was not reliable.

Figure legend

Figure 1: Flow diagram of sample collection and results. Newborn screening dried blood spot sample collection, and primary results for Congenital Hypothyroidism (CH), Sickle Cell Anaemia (SCA) and Sickle Cell Trait.

Contributorship statement

Bibi A. Alladin, Pheona Mohamed-Rambaran, Vijay Grey and Andrea Hunter developed the proposal.

Bibi A. Alladin and Pheona Mohamed-Rambaran provided resources and supervision for sample collection, shipment and follow up of results locally, including reporting and patient contact and completed the final manuscript

Bibi A. Alladin secured funding.

Pranesh Chakraborty, Matthew Henderson, Jennifer Milburn, Laure A. Tessier contributed to the methodology, provided resources for analyzing samples, interpretation of the results and assisted with compilation of the manuscript.

Competing interests

None

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Georgetown Public Hospital Corporation (GPHC) paid for the shipment of samples to NSO weekly and provided human resources for sample collection.

Data sharing statement

Extra data is available and can be accessed by emailing Pheona Mohamed-Rambaran at pheonar@yahoo.com.

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Dr. Jennifer McKnight provided information on NBS in the Caribbean and has volunteered to assist with implementation of NBS in Guyana.

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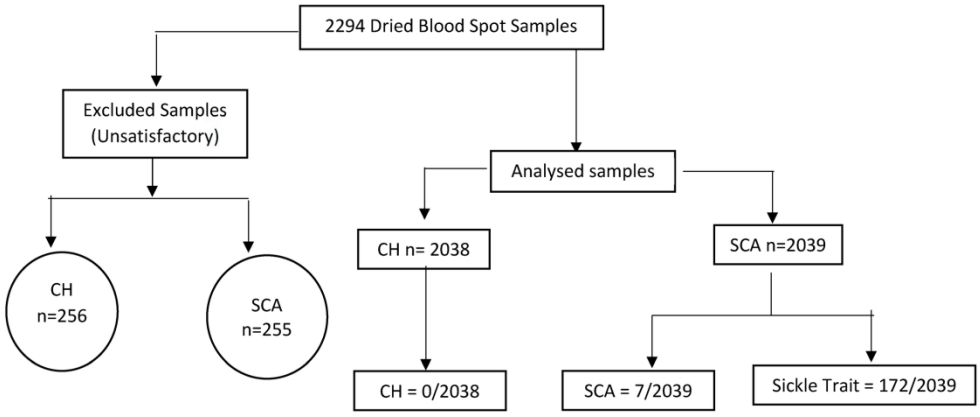

Figure 1: Flow diagram of sample collection and results. Newborn screening dried blood spot sample collection, and primary results for Congenital Hypothyroidism (CH), Sickle Cell Anaemia (SCA) and Sickle Cell Trait.

Flow diagram of sample collection and results. Newborn screening dried blood spot sample collection and primary results for Congenital Hypothyroidism (CH), sickle Cell Anaemia (SCA) and Sickle Cell Trait.

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A cross-sectional prospective feasibility study of newborn screening for sickle cell anemia and congenital hypothyroidism in Guyana

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Abstract

Introduction: Newborn screening (NBS) is a test done shortly after birth to detect conditions that cause severe health problems if not treated early. An estimated 71% of babies worldwide are born in jurisdictions that do not have an established NBS program. Guyana currently has no NBS program and has established a partnership with Newborn Screening Ontario (NSO) to initiate screening. Objectives: To assess the feasibility of implementing a NBS program in Guyana for congenital hypothyroidism (CH) and hemoglobinopathies (HBG) and to report on screen positive rates and prevalence (Hardy-Weinberg equilibrium (HWE) for CH and HBG.

Methods: Term, healthy Guyanese infants were evaluated (with consent) using heel prick dried blood spots (DBS) shortly after birth (closer to 24 hours of life). DBS samples were analysed at NSO. Screening test for CH was done using a human thyroid-stimulating hormone (hTSH) assay. Mean hTSH levels between the Guyanese sample and the Ontarian population were compared using

Student's T test with an alpha of 0.05. Screening test for SHBG was performed with a cation-exchange high-performance liquid chromatography (HPLC).

Results: The pilot was conducted from June 6th, 2016, to September 22nd, 2017. GPHC recruited 2,294 mothers/infants. Screen positive rate for CH in our sample was 0.0% (0/2,038 infants). Mean TSH levels in Guyanese samples (1.7 µU/mL blood) was noticed to be significantly different than in the Ontarian population (4.3 µU/mL blood) ($p < 0.05$). Screen positive rate for Sickle Cell Anaemia (SCA) in our sample was 0.3% (7/2,039 patients), and the carrier rate was 8.41% (172/2,039patients). Using the HWE, the SCA frequency (S allele frequency) ^{2} is $0.049^2 = 0.002$

Conclusion: NBS for CH and SCA in Guyana could be beneficial. Future work should focus on conducting larger pilots which could be used to inform diagnosis and treatment guidelines for Guyanese people.

Article Summary

Strengths and limitations of this study

- This first-time study on newborn screening in Guyana provides baseline data on sickle cell anemia and thyroid hormone levels.
- The findings can be used by policy makers to justify future work, including policies for newborn screening in Guyana.

Limitations

- The absence of previous studies prevented comparison and interpretation of thyroid hormone levels in the neonates.
- The small sample size and single centre study prevents generalization of findings to the Guyanese population.
- Difficulties were encountered with following up of participants with borderline or positive results due to their geographic locations.

Introduction

Newborn screening (NBS) is a screening test done shortly after birth to detect conditions that cause severe health problems if not treated early.[1]NBS programs have been existing for over 50 years in developed countries.[2]It allows clinical providers to identify infants at high risk for those conditions, provide diagnostic testing, and initiate treatment to avoid severe outcome and to prevent death.[3]Conditions included in NBS need to have a relatively high prevalence and their testing and treatment need to be simple, inexpensive and effective.[2]While the number of conditions included in NBS panels varies across jurisdictions, the most advanced laboratory settings give NBS programs the capability for screening for over 50 conditions.[3]

Centralization of testing and advanced analytical methods such as tandem mass spectrometry which has increased the sensitivity and rapidity in evaluation have contributed to the progress made with screening neonates .[4]In lower- and middle-income jurisdictions, barriers such as lack of resources and infrastructures have limited the development of NBS programs.[4]Approximately 71% of babies worldwide are born in a jurisdiction that does not have an established NBS program.[2] As such, the majority of infants born worldwide with diseases for which NBS exists do not get diagnosed and treated early. This leads to significant mortality, morbidity and economic burden of NBS-eligible diseases in jurisdictions where NBS is not developed. [2]

Guyana currently has no NBS program and has established a partnership with Newborn Screening Ontario (NSO) to initiate screening. NSO was established in 2006 and coordinates NBS for the province of Ontario, Canada. NSO screens over 140,000 newborns per year for twenty-nine targeted diseases by collecting and analyzing dried bloodspot (DBS) samples in the first days of life. Congenital hypothyroidism (CH) and sickle cell anaemia (SCA) are two relatively common conditions,

which are good candidates for NBS and offered by NSO. In the Guyanese population, the prevalence of CH and SCA are unknown but there is evidence that they both have a significant public health burden based on the number of paediatric patients requiring care with SCA and hypothyroidism as outpatients and inpatients, making these two conditions eligible for a NBS pilot in Guyana.

CH is an endocrine condition that can result in growth deficiency and severe intellectual disability if left untreated.[1]It is one of the most common preventable cause of intellectual disability with an incidence in newborns between 1:2000 and 1:4000, and as high as 1:600 newborns in iodine deficient areas. [1]CH is most often permanent and is caused by an abnormality in thyroid gland development or a defect in thyroid hormonogenesis. [5]In some cases, the condition is transient, in which can be due to the transplacental passage of maternal medications, maternal blocking antibodies, iodine deficiency or excess. [5]Treatment for CH is effective, inexpensive and easy to manage for families.[1]Infants diagnosed with CH near birth can start treatment and avoid any growth delay or intellectual disability and develop normally.[1]However, when CH patients are identified later, treatment does not suffice to eliminate developmental and growth delays resulting from CH, which underlines the importance of NBS for CH. [1]CH was added to NBS programs starting in the mid-1970s, and has shown to be effective in reducing morbidity, mortality and the economic burden of CH: the jurisdictions screening for CH have eliminated neuro-developmental impairments resulting from CH.[2]Since establishing NBS for CH, Cuba, Puerto Rico and the US Virgin Island have been screening more than 95% of their population for CH and reported a birth incidence ranging from 1: 3,600 and 1: 9,526. [2]In Brazil, the CH prevalence has been reported as 1: 2,259. [2]The CH prevalence in Guyana remains unknown.

In Guyana, SCA is among the leading causes for admission to pediatric wards. During a one-year study, it accounted for 102 of 1380 admissions.[6]SCA is a group of autosomal recessive inherited conditions that affects the formation of haemoglobin and the functioning of red blood cells. The gene mutations responsible for SCA are most common in populations of African, South Asian, Middle

Eastern and Mediterranean origins.[7]SCA is one of the most common genetic conditions worldwide. The global birth prevalence of homozygous sickle cell anaemia is estimated to be 111.91 per 100,000 live births with disparities by region. Africa has a birth prevalence of 1125.49 per 100,000 compared with 43.12 per 100,000 in Europe.[8]

SCA is a global health public concern, a survey conducted by the World Health Organization (WHO) reported that haemoglobin disorders such as SCA are a public health concern for 71% of the 229 countries surveyed, which represent 89% of births worldwide. [9]

While the prevalence of SCA in Guyana in unknown, data is available for some Caribbean countries through NBS. Jamaica has the highest prevalence of SCA at 0.65%, followed by Grenada (0.63%), Haiti (0.58%) and Tobago (0.57%). Trait prevalence was highest in Jamaica at 14% and St. Vincent and Grenadines (15%), followed by St. Lucia (13.8%), Haiti (13.5%) and Tobago (13.2%). [10]

Sickle Cell Disease (SCD) patients experience a wide range of adverse outcomes, including, but not limited to: anaemia, stroke, ischemic organ damage, pain crises, chronic respiratory disease, recurrent infections, and death. [11]. In 2008, the United Nations (UN) recognized SCA, a form of SCD , as a public health concern and urged relevant parties to strengthen health systems and primary care delivery for SCA. [12] Since this declaration, many jurisdictions have recognized the need for SCA NBS in order to enable early identification and treatment of SCA affected infants. [12] Several studies have concluded that NBS for SCA and early treatment for SCA leads to reduced morbidity and mortality. Notably, a study conducted in the Republic of Benin found that establishment of a NBS for SCA has led to a mortality rate 10 times lower for children under 5 years of age.[13] In North America, SCA mortality for children under 5 years of age has been almost eliminated thanks to early identification and treatment from NBS.[14]

The Georgetown Public Hospital Corporation (GPHC) partnered with NSO to conduct a pilot study of NBS for CH and SCA in Guyana. Given the infrastructure challenges for establishing and delivering NBS in Guyana, partnering with an established NBS program could help overcome obstacles and help

provide data that support the implementation of a NBS program, such as disease prevalence and the NBS test's positive predictive values in the Guyanese population.

Objectives

The objectives of this pilot NBS study were:

- To assess the feasibility of implementing a NBS program in Guyana for CH and HBG
- To assess the potential benefits of implementing a NBS program in Guyana for CH and HBG
- To report on screen positive rates for CH and HBG (sickle cell disease and traits) for the Guyanese population
- To report on HBG prevalence in Guyana based on NBS results and using the Hardy-Weinberg equilibrium (HWE)

Methods

Study Design

This pilot project was a prospective descriptive study at done at GPHC from June 6th, 2016 to September 22nd, 2017.

Inclusion criteria

Term, healthy babies (gestational age of 37 weeks or later) born at GPHC during the study period whose mothers provided written consent after being briefed on the study.

Exclusion criteria

Babies with sepsis, and those admitted to the neonatal intensive care unit were excluded since their mothers were potentially under undue stress and may not have consented. Additionally, the sampling of these babies may have created additional exposure to personnel who can introduce

infection and further compromise their immune system. Further, sick newborns can have abnormal TSH due to their illness which may not be indicative of CH and could have cause a bias in the findings.

Sample size

The sample size was calculated to be 3250 neonates based on the number of babies born annually, which represented the duration of funding.. Guyana has an average of 15,000 live births per year with 22% being born at GPHC. It was not feasible to include rural hospitals in this pilot study.

Sample Collection and handling

Newborn babies were tested after mothers were given a written document and a brief discussion of the purpose of the research, the risks and benefits of being tested and the potential complications from a heel prick DBS sample collection. A written consent form was provided to mothers for signature. Some mothers requested that the fathers' consent as well. Only babies whose parents consented were included in the study. Data was coded with a unique identifier.

A dried bloodspot (DBS) sample was collected by trained laboratory technicians via heel prick and using a filter card shortly after birth (closer to 24 hours of life). These samples were stored at the Medical Laboratory at GPHC according to standard protocols for storing DBS. DBS filter cards were air shipped (at room temperature) weekly to NSO for analysis, with a shipping turnaround time of three days.

Analysis

Samples were analysed for CH and SCA on the day of receipt by NSO, and the NBS results were sent from NSO to GPHC within 14 days of samples receipt. NBS results were categorized as: screen positive (higher probability that the infant is affected by the disease and confirmatory diagnosis testing is needed), screen negative (low probability that the infant is affected by the disease and no

follow up is needed), or unsatisfactory (the sample quality did not permit the NBS test). Confirmatory testing and follow-up care were planned at GPHC.

CH

The screening test for CH was done using a human thyroid-stimulating hormone (hTSH) assay. The neonatal hTSH assay is a solid phase, two-site fluoroimmunometric assay based on the direct sandwich technique. Specimens containing hTSH are reacted with anti-hTSH IgG, coated on the wells of the microtitre plates, and europium-labelled anti- hTSH IgG (Eu tracer). The wash buffer elutes hTSH from the DBS. The enhancement solution dissociates the europium ions from the labeled antibody where they form highly fluorescent chelates. The fluorescence measured in each well is proportional to the concentration of hTSH in the sample. If the initial hTSH level in a sample was found to be greater than or equal to 13 µU/mL of blood, a confirmatory hTSH measurement was made using the same technique outlined above. If the confirmatory hTSH level was found to be greater than or equal to 17 µU/mL of blood, the newborn was considered screen positive for CH. Otherwise, the patient is categrorized to be screen-negative for CH. Mean hTSH levels between the Guyanese sample and the Ontarian population were compared using Student's T test with an alpha of 0.05.

SCA

The screening test for SCA was performed with a cation-exchange high-performance liquid chromatography (HPLC) used to separate the different variants of hemoglobin present in a DBS elutes (reconstituted DBS). Using a two dual-piston pump, the ionic strength of the buffer is increased, encouraging the more strongly retained hemoglobin to elute from the column. Each hemoglobin has a characteristic retention time. The instrument quantifies the amount of hemoglobin variants present in the sample. These include adult hemoglobins (A, A2) which are the most common types in the normal population; fetal hemoglobins (F, F1) and minor hemoglobins (E,

S, C). Together, the quantitative hemoglobin results (in the form of peaks in the chromatogram) are interpreted by a clinician who assigns the chromatogram a single *hemoglobin pattern* determination. The hemoglobin pattern determination was used to determine if the newborn was screen negative or screen positive for SCA. This NBS tests allows for identification of carriers of SCA.

S allele frequency was calculated using the below equation (equation 1)

(1) S allele frequency = (number of carriers) + (2 x (number of screen positive) / (number of infants screened x 2)

SCA prevalence in Guyana was estimated using a population of approximately 746,955 (according to a census conducted in 2012 conducted by the Bureau of Statistics of Guyana). [13] The Bureau of Statistics estimated projected births be between 13,963 and 15,864 between 2000 and 2025 in Guyana was used to calculate the birth prevalence of SCA. [15]

Outcome measures

Primary outcomes were to report on screen positive rates for CH and HBG for the Guyanese population using Hardy-Weinberg equilibrium (HWE) and percent positivity rates. The HWE is commonly used to estimate the frequency of a phenotype in a population, for example a recessive condition like SCA, because sample sizes in cross-sectional studies are usually too small to allow for a precise estimate. [16,9] For CH, mean hTSH levels and mean difference between the Guyanese sample and the Ontarian population were compared using Student's T test with an alpha of 0.05.

Secondary outcome was to assess the feasibility of CH and SCA NBS as a public health program in Guyana using *Wilson-Jungner criteria* as the outcome measure (as shown in Table 1).

Table 1: Wilson-Jungner principles [17]

Principle	Principle met?
1. The condition sought should be an important health problem.	Yes

2. There should be an accepted treatment for patients with recognized diseases	Yes
3. Facilities for diagnosis and treatment should be available	Available but was not assessed in this pilot. GPHC can diagnose and treat SCA and CH.
4. There should be a recognizable latent or early symptomatic phase	Yes
5. There should be a suitable test or examination	Yes
6. The test should be acceptable to the population	Yes
7. The natural history of the condition, including development from latent to declared disease, should be understood	Yes
8. There should be an agreed policy on whom to treat as patients	Draft document awaiting final approval by Ministry of Health, Guyana
9. The cost of case-finding (including diagnosis and treatment of patients diagnosed should be economically balanced in relation to possible expenditure on medical care as a whole	Yes
10. Case-finding should be a continuing process and not a "once and for all" project	Yes–policy brief for implementation of NBS was presented to the Pan American Heath Organization (PAHO) Guyana office in 2020 for implementation of routine NBS. No further work on this due to COVID-pandemic.

Research Ethics

This study was reviewed and approved by the Guyana Ministry of Public Health Institutional Review Board (IRB #225) and by the Children's Hospital of Eastern Ontario Research Ethics Board (REB Protocol No: 17/210X).

Patient and Public Involvement

No patients or member of the public were included in the design of this study, patients were included in the data collection phase and in the reporting of results that needed follow-up. Patients were recruited as the sample population after sensitization of their mothers.

Results

Pilot experience and feasibility

The pilot was conducted from June 6th, 2016, to September 22nd, 2017. GPHC recruited 2,294 mothers/infants. There were no reported harms to babies who were assessed. All samples were successfully shipped to NSO. Confirmation of diagnosis of the screen positive neonates was not obtained since telephone numbers provided were unreachable and mothers failed to bring in their child, so this report is solely on the screen positive rate for CH and SCA, and on the estimated prevalence of SCA assuming HWE. Of note, one mother followed up and her child was confirmed as SCA.

CH

Of the 2,294 received samples, 256 were excluded from CH NBS testing as they were determined unsatisfactory for testing (Figure 1). The most common reason for exclusion was an insufficient blood quantity of the sample. The screen positive rate for CH in our sample was 0.00% (0/2,038 infants). Mean TSH levels in Guyanese samples (1.70 µIU/mL blood) was significantly different ($p < 0.05$) than in the Ontarian population (4.30 µIU/mL blood).

SCA

Of the 2,294 received samples, 255 were excluded of SCA NBS testing as they were determined unsatisfactory for testing (Figure 1). The most common reason for exclusion was insufficient blood quantity of the sample. The screen positive rate for SCA in our sample was 0.30% (7/2,039 patients), and the carrier rate was 8.41% (172/2,039 patients). As we were unable to obtain follow-up diagnostic data for all the screen positive infants, the true positive rate and the positive predictive value of this SCA NBS test in the Guyanese population could not be ascertained. However, as this NBS test identifies SCA-affected infants as well as carriers, the allele frequency for SCA in the study

sample and estimate of SCA prevalence in the Guyanese population using the HWE, assuming sample is representative of the population in terms of allele frequency, could be deduced. Table 2 presents the genotype and S allele frequencies in the study sample; the S allele frequency being represented by equation 1 in methods section:

Table 2: SCA genotype and S allele frequencies in the study sample

AA frequency – screen negative (%)	AS frequency – carriers (%)	SS frequency – screen positive	S allele frequency
51.35	9.07	0.34	0.049

Using the HWE, the SCA frequency is (S allele frequency) ^{2} = 0.049 ^{2} = 0.002.

Using the projected estimate birth value for 2020 of 15,126 births, it can be estimated that the birth prevalence of SCA in Guyana is 30 births per year (0.2%).

CH and SCA as NBS candidate diseases in Guyana as per Wilson-Jungner criteria

To further assess the suitability of CH and SCA NBS as a public health program in Guyana, mapping of Guyana's experience was done onto the Wilson-Jungner criteria from Table 1. These criteria were established in 1968 in a report for the World Health Organization[18] and have been developed as an evaluation framework to determine the eligibility of a given condition and its test for a screening program. The Wilson-Jungner principle have been widely used as a checklist to establish NBS programs.[17] The principles acknowledge the technical characteristics of a given NBS test as well the significance of political, economic, social and health issues of an NBS test or program.[17] Table 1 presents the 10 Wilson-Jungner principles and whether each principle was met in this pilot study. Seven out of the ten principles were met in the study. Principle 3 was not assessed in this pilot but is met locally. Principles 8 and 10 were being addressed prior to the pandemic and is currently on hold due to prioritization of resources.

Discussion

A pilot study of NBS in Guyana was conducted in which a remote laboratory performed the screening tests. The pilot has shed light on the feasibility and potential benefits of NBS for CH and SCA in the Guyanese population. This pilot study also provided an estimate of the SCA prevalence and birth prevalence in Guyana.

There were no screen positive infants for CH in the sample population, but the researchers are familiar with cases of CH in Guyana. Small sample population of 2294 was responsible for zero positivity for CH as incidence ranges from 1:2000 to 1:4000. At least 4000-5000 babies will need screening for one positive sample. [1] Currently, children are evaluated for hypothyroidism in Guyana if there is a high index of suspicion such as developmental delay. Late diagnosis and intervention have adverse prognostic factors for these children. Further research is needed to determine the prevalence of CH in newborns and the potential causes of the mean TSH levels difference between Guyana and Ontario, and whether TSH cut-offs for CH NBS in Guyana must be adjusted accordingly. It would notably be important to determine whether the shipment time and conditions (temperature, humidity) from Guyana to Ontario, Canada affect TSH levels in the DBS, or if mean TSH levels are different in the Guyanese population due to other factors specific to this population. It is possible that some infants in the study sample are in fact affected by CH with TSH levels higher than the mean TSH level for the sample population. Diagnosis of CH could not have been confirmed due to lack of follow up data. Confirming diagnosis would further help determine if the TSH cut-off for CH NBS tests in Guyana should be different than the one used in Ontario by NSO. Currently, we are unable to comment on recommending CH as a priority NBS in Guyana.

The sample's screen positive rate for SCA (0.3%), as well as the estimated SCA allele frequency and birth prevalence of SCA in Guyana assuming HWE (0.2%) are lower than what has been reported in some Caribbean jurisdictions.[10] This may be due to small sample size, insufficient statistical power

and insufficient external validity of the study sample, and centralized testing at GPHC without having a good representation of the rural areas in the sample population as SCA is higher in Afro-Caribbean people. To our knowledge, this is the first report of an estimated prevalence and birth prevalence of SCA in Guyana, and further research is needed to clarify these findings. The estimated birth prevalence from this study suggests that about thirty newborns per year in Guyana are affected by SCA, which indicates that Guyana may benefit from SCA NBS. Guyana recently concluded a draft document for the management of SCA and Thalassemia and there is an increase in speciality service for this population. Guyana has a significant East Indian ancestry with genetic susceptibility to Thalassemia, this population may benefit from HGB screening that may identify beta Thalassemia.

The eligibility of CH and SCA NBS in Guyana is further supported by our assessment of this pilot's experience using the Wilson-Jungner principles. Only three of the ten principles have not been met in this study. These three unmet principles were out of the study's scope and could be established in future work. Principle three (3), *Facilities for diagnosis and treatment should be available*, Principle eight (8), *There should be an agreed policy on whom to treat as patients*, and Principle ten (10), *Case-finding should be a continuing process and not a "once and for all" project,* could be met through some policy work in Guyana. Bibi A. Alladin has developed a policy brief for the implementation of NBS for HGB in Guyana under the guidance of Pan American Health Organization (PAHO).

Overall, our experience suggests that NBS for CH and SCA in Guyana could be beneficial, and that it is feasible to establish a program by shipping DBS samples to a remote NBS lab for analysis while Guyana builds capacity for local testing. Over the last three years, Guyana has dispatched more doctors to the rural areas as the medical capacity continues to grow, this may help to mitigate the challenges with following up of screen positive samples from rural areas through the network of primary health care.

Future work should focus on conducting larger pilots that would allow more diagnostic data to be collected, which in turn would allow a more accurate estimate of positive predictive values of the NBS tests and a better estimation of the mortality and morbidity reduction in Guyana if NBS is implemented. Additionally, these finding could be used to inform diagnosis and treatment guidelines for Guyanese people.

Limitations

Lack of access to testing facilities in Guyana was a major hinderance to the start of this project until NSO became involved. Only children born centrally were included in the study due to lack of resources and access to rural areas. Sample population may not be a true representation of the Guyanese population distribution. There were no positive cases of CH due to small sample size as CH prevalence ranges from 1:2000 to 1:9000. Larger studies with more robust methods for diagnostic follow up are needed to establish prevalence of CH in newborns in Guyana, limited funding did not permit a larger study at this time.

Sample rejection was 11%, this was due to lack of familiarity of DBS as a routine method of sample collection. Technicians were trained for DBS, but skill set varied among technicians. This further reduced our sample size for analysis.

We were unable to follow up most of the screen positive cases for SCA despite many attempts as these cases were all from the rural areas and contact information was not reliable.

Figure legend

Figure 1: Flow diagram of sample collection and results. Newborn screening dried blood spot sample collection, and primary results for Congenital Hypothyroidism (CH), Sickle Cell Anaemia (SCA) and Sickle Cell Trait.

Contributorship statement

Bibi A. Alladin, Pheona Mohamed-Rambaran, Vijay Grey and Andrea Hunter developed the proposal.

Bibi A. Alladin and Pheona Mohamed-Rambaran provided resources and supervision for sample collection, shipment and follow up of results locally, including reporting and patient contact and completed the final manuscript

Bibi A. Alladin secured funding.

Pranesh Chakraborty, Matthew Henderson, Jennifer Milburn, Laure A. Tessier contributed to the methodology, provided resources for analyzing samples, interpretation of the results and assisted with compilation of the manuscript.

Competing interests

None

Funding

Grant number: Not applicable

This project was funded by Guyana Bank for Trading and Industry (GBTI) through a public-private partnership. Funds were used to purchase supplies for sample collection and for testing.

Georgetown Public Hospital Corporation (GPHC) paid for the shipment of samples to NSO weekly and provided human resources for sample collection.

Data sharing statement

Extra data is available and can be accessed by emailing Pheona Mohamed-Rambaran at pheonar@yahoo.com.

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Dr. Jennifer McKnight provided information on NBS in the Caribbean and has volunteered to assist with implementation of NBS in Guyana.

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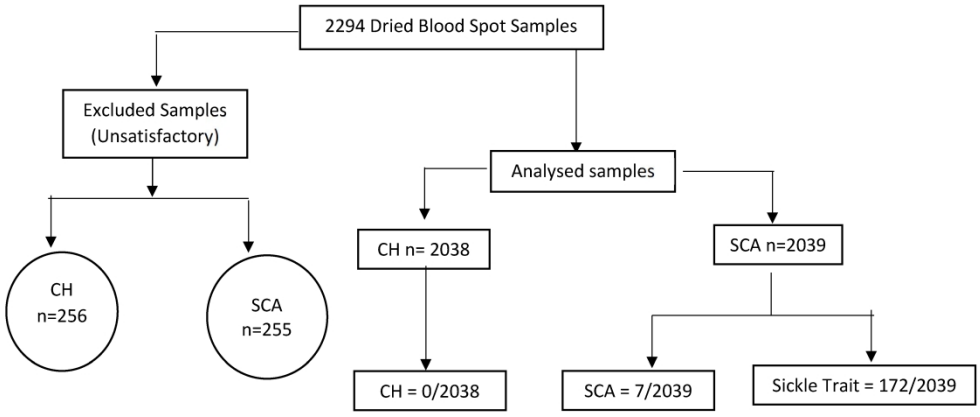

Figure 1: Flow diagram of sample collection and results. Newborn screening dried blood spot sample collection, and primary results for Congenital Hypothyroidism (CH), Sickle Cell Anaemia (SCA) and Sickle Cell Trait.

Flow diagram of sample collection and results. Newborn screening dried blood spot sample collection and primary results for Congenital Hypothyroidism (CH), sickle Cell Anaemia (SCA) and Sickle Cell Trait.

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