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Strengthening laboratory capacity through the surveillance of rotavirus gastroenteritis in Central Africa: the Surveillance Épidémiologique en Afrique Centrale (SURVAC) Project

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Abstract

OBJECTIVES—The goal of the SURVAC pilot project was to strengthen disease surveillance and response in three countries; Cameroon (CAE), Democratic Republic of the Congo (DRC) and Central African Republic (CAR).

METHODS—Seven laboratories involved in rotavirus surveillance were provided with equipment, reagents and supplies. CDC and WHO staff provided on-site classroom and bench training in biosafety, quality assurance, quality control (QC), rotavirus diagnosis using Enzyme Immunoassay (EIA) and genotyping of rotavirus strains using the Reverse Transcription Polymerase-chain reaction (RT-PCR). All laboratory data were reported through WHO/AFRO.

RESULTS—Twenty-three staff members were trained on RT-PCR for rotavirus genotyping which was introduced for the first time in all three countries. In CAE, the number of samples analysed by EIA and RT-PCR increased tenfold between 2007 and 2013. In DRC, this number increased fivefold, from 2009 to 2013 whereas in CAR, it increased fourfold between 2011 and 2013. All laboratories passed WHO proficiency testing in 2014.

CONCLUSION—Laboratory capacity was strengthened through equipping laboratories and strengthening a subregional laboratory workforce for surveillance of rotavirus gastroenteritis. Each of the three countries generated rotavirus surveillance and genotyping data enabling the mapping of circulating genotypes. These results will help monitor the impact of rotavirus vaccination in these countries.

Keywords

laboratory; capacity; Central Africa; rotavirus

Introduction

Significant challenges to an effective implementation of laboratory surveillance of diseases in the Central African region include the lack of trained workforce, shortages of reagents and supplies and inadequate infrastructure. SURVAC (Surveillance Épidémiologique en Afrique Centrale) was a surveillance demonstration project implemented from 2009 to 2014 in three countries in the Central African subregion: Cameroon (CAE), The Democratic Republic of the Congo (DRC) and Central African Republic (CAR) (Figure 1). This subregion was chosen because of it epidemiologic importance in terms of disease outbreaks and emerging infections and has significant gaps in generating and using epidemiological data to guide decision-making for disease control. SURVAC is a French acronym that translates to Strengthening Surveillance and Response in Central Africa.

The three overarching goals of SURVAC were to strengthen surveillance and response capacity and quality through training and infrastructure improvements; implement a quality surveillance and response program for vaccine preventable diseases/syndromes, including laboratory capacity, networks and data management systems; strengthen communications

infrastructure and develop capacity for advocacy to ensure that these efforts are assumed by the Ministries of Health (MoH) in the target countries. The project encompassed four components: epidemiology, laboratory, the Field Epidemiology and Laboratory Training Programme (FELTP) and Information Communication Technology (ICT).

To fulfil the surveillance goals of the countries and to prepare for new vaccine expected to be introduced in these countries, SURVAC strengthened surveillance for rotavirus gastroenteritis and paediatric bacterial meningitis first and later enhanced laboratory support for existing surveillance for measles and rubella and other priority outbreak prone diseases.

The World Health Organization (WHO) recommends inclusion of rotavirus vaccination of infants in all national immunisation programmes [1]. Before such rotavirus vaccination can be implemented, these countries need to provide data concerning burden of rotavirus disease to drive policy decisions. Previous studies showed that the genetic evolution or changes in circulating virus population may have a potential impact on vaccination programmes [2]. Thus, it is important to collect rotavirus genotype information before the introduction of the rotavirus vaccine to help monitor the impact of the vaccine in the future. Rotavirus strain genotyping is commonly based on a dual nomenclature using both VP4 and VP7 genes [3], using a reverse transcription-polymerase chain reaction (RT-PCR) technique. Therefore, it was critical to establish RT-PCR technique in SURVAC countries to characterise circulating rotavirus strains. This article outlines challenges found in Central Africa prior to SURVAC support, describes the implementation strategy and progress made within the 5 years of implementation of the SURVAC project.

Methods

Laboratory referral system for rotavirus surveillance

In 2006, WHO/AFRO established a programme, the African Rotavirus Surveillance Network, to generate country-specific data on the burden of rotavirus diarrhoea, and characterise and document the circulation of rotavirus strains in the region. This included laboratory capacity building at national level to support confirmation of rotavirus cases by enzyme immunoassay (EIA) technique and a proficiency testing (PT) programme for external quality assessment [4].

The MoH nominated laboratories to be involved in rotavirus surveillance. This included a laboratory located in the paediatric sentinel site hospital and a national (central level) laboratory. Cases were enrolled at the sentinel site, and stool samples were collected and tested by EIA for Group A rotavirus antigen at the sentinel site laboratory. Positive samples were then shipped to the national laboratory for confirmation by EIA and quality control (QC). Each year 50 rotavirus-positive samples were subsequently sent to the Regional Reference Laboratory (RRL) in South Africa for genotyping [5]. Cameroon and DRC started rotavirus surveillance in 2007 and 2009, respectively, as part of the African Rotavirus Surveillance network.

The subsequent establishment of SURVAC project added new momentum, financial resources and techniques to the national laboratory activities for rotavirus testing. National

laboratories were supported to assume a greater role in providing rotavirus genotyping using molecular biology, a multiplex RT-PCR technique, to meet expanding national rotavirus surveillance in anticipation of rotavirus vaccine introduction. The RT-PCR technique established in the national laboratories for rotavirus genotyping was equivalent to the technique used in the RRL.

Laboratory assessment

An on-site assessment of laboratories capacity in each country, using a standardised assessment tool, was performed first. The MoH of each country provided a list of laboratories that could potentially be involved in vaccine preventable disease surveillance activities. A multi-institutional team composed of members of the United States Centers for Diseases Control and Prevention (CDC), the World Health Organization (WHO) and the MoH of the corresponding country assessed the laboratory capacity and systems in DRC, CAE and CAR.

In CAE, five laboratories in Yaoundé and Douala were assessed. In Yaoundé, this included the Centre Pasteur de Yaoundé, The Mother and Child Center, the Lanacome Laboratory and the Laboratoire National de Santé et d'Hygiene Mobile, and in Douala, the Laquintinie Hospital laboratory.

In DRC, six laboratories were assessed, five in Kinshasa and one laboratory in Lubumbashi. In Kinshasa, these were the Institut National de Recherches Biomedicales (INRB), the Hopital Pédiatrique Kalembelembe, the Hopital Kingasani, as well as laboratories for disease-specific programme for Tuberculosis, HIV and Malaria. In Lubumbashi, the laboratory of the Hopital General Sendwe was assessed.

In CAR, the assessment of five laboratories was limited to the town of Bangui. The laboratories assessed were the Pasteur Institute of Bangui, The Laboratoire National de Santé Publique, the HIV Laboratory, the Blood Bank Laboratory and the Clinical Laboratory of the Complex Pediatrique de Bangui Hospital.

In addition, the department responsible for co-ordinating laboratory activities at the MoH of three countries was reviewed using a specific standardised questionnaire to ascertain ability to support and sustain this project.

To guide these assessments, an assessment tool developed by WHO [6] was used. Each laboratory was visited, and interviews were conducted with laboratory personnel. The personnel of the section in charge of laboratory activities at the MoH were also interviewed.

Topics assessed included the structure and organisation of laboratories, specimen collection and transportation, biosafety/biosecurity, QC, the role of the laboratory in diseases surveillance, diagnostics capacity, funding and procurement of laboratory equipment, reagent and supplies, laboratory data management, training of laboratory personnel and communication.

Procurement of equipment, reagent and supplies

Laboratory equipment was procured through the WHO/AFRO procurement system and delivered to the country. Some equipment, not available in WHO/AFRO's order list, was provided by the CDC. Reagents and supplies were first provided by the CDC; however, since June 2012, they have been provided by WHO/AFRO and CDC until the final year of the project, when the MoH of each country assumed responsibility.

Training of laboratory personnel

All laboratory personnel involved in the surveillance of rotavirus were trained first during a subregional workshop held in DRC in 2010, attended by the personnel from all three SURVAC countries [7]. This initial training in 2010 included 15 laboratory staff as follows: (i) three staff from CAE; two from the Mother and Child Center laboratory and one from the Laboratoire National de Sante et d'Hygiene Mobile, (ii) eight staff from DRC; four from the INRB laboratory, two from the Hopital pediatrique Kalembelembe, one from Hopital Kingasani and one from Hopital General Sendwe and (iii) four staff from CAR; two from the Pasteur institute of Bangui, one from the Complex Pediatrique de Bangui and one from the Laboratoire National de Sante Publique. The main topic was on the diagnosis and characterisation of rotavirus strains using RT-PCR followed by other topics such as biosafety, quality assurance and QC, specimen handling and transport and EIA techniques. Additional onsite bench training was provided at least once per year in each home laboratory with regular (twice per year) follow up supervision. During these on-site trainings between 2011 and 2014, eight more personnel were trained; four in CAE from the Mother and Child Center laboratory, one in DRC from Hopital paediatric Kalembelembe and three in CAR with two from the Pasteur Institute of Bangui and one from the Complex Pediatrique de Bangui. In 2013, two laboratory personnel, one from the Mother and Child Center laboratory in CAE and one from the INRB in DRC were trained at the CDC in Atlanta in more advanced molecular biology techniques such as sequencing, sequence analysis and phylogenetic analysis. To improve the integration of laboratories activities, laboratory personnel were also trained on the diagnosis of other vaccine preventable diseases such as measles and rubella using RT-PCR techniques.

Laboratory analyses of rotavirus samples

Stool samples were collected in sentinel sites hospital from all children less than 5 years old who met the WHO case definition of gastroenteritis [8]. EIA testing and genotyping multiplexed RT-PCR were performed as previously described [9]. Briefly, stool samples were first screened for group A Rotavirus antigen by EIA at the sentinel site laboratory. Aliquots were then stored at —20°C before being transported to the central level laboratory where results were confirmed by EIA and genotyping assays were performed, using a multiplex RT-PCR technique. Rotavirus strain genotyping is commonly based on a dual nomenclature using both VP4 (P) and VP7 (G) genes [3]. Samples subjected to genotyping were subsequently confirmed at the CDC, Atlanta, USA, for QC.

Proficiency testing on rotavirus diagnosis by EIA and genotyping by RT-PCR techniques

SURVAC laboratories participated in the PT programme organised by CDC and WHO/AFRO. Through this programme, 8–10 PT samples are sent to the participating laboratories once per year. The laboratories were asked to screen the samples for group A rotavirus antigen by EIA and to identify their genotype (G and P) using RT-PCR techniques and results are sent back for evaluation. The passing score for each portion (EIA and RT-PCR) was 80%.

Results and discussion

Laboratory assessment

The implementation of the SURVAC project began in 2009 with the assessment of laboratory systems in the three SURVAC counties: CAE, DRC and CAR. The outcome of the assessment conducted at the beginning of the implementation of SURVAC project indicated that laboratory services and infrastructure were very weak throughout SURVAC countries. In most laboratories, much equipment was broken or could not be used due to the lack of proper maintenance. Reagents and supplies were not always available and this severely affected laboratory analyses. This situation is common in many developing countries and has been described in Africa [10, 11] and the Caribbean [12].

With regard to rotavirus surveillance, the laboratories designated by the MoH to play a role in the surveillance of rotavirus were: in CAE, the laboratory of the Mother and Child Center was selected to play the roles of both the central level laboratory and the sentinel site. In DRC, the Institut National de Recherches Biomedicales (INRB) was selected as the central level laboratory. The Hopital Pediatrique Kalembelembe, the Hopital Kingasani and the Hopital General Sendwe were the three sentinel sites selected. In CAR, the Pasteur Institute of Bangui was selected as the central level laboratory and the Complex Pediatrique de Bangui was chosen as the sentinel site laboratory.

Before SURVAC, the level of rotavirus testing capacity within SURVAC countries varied. In CAE and DRC where rotavirus surveillance started in 2007 and 2009, respectively, rotavirus samples analysis was limited to EIA testing only and due to resource constraints genotyping was performed at the RRLs in South Africa and Ghana [4]. In CAR, there was no surveillance system for rotavirus before the SURVAC project. Although CAE and DRC had attended annual training workshops conducted by RRL in South Africa that included RT-PCR techniques, none of the SURVAC countries had established molecular testing for rotavirus genotyping by RT-PCR in their laboratories. For several years, DRC and CAR had been conducting PCR testing for other diseases.

The results of the assessment were used to develop a working plan for improving the country’s surveillance, laboratory and response. In anticipation of the introduction of rotavirus vaccine, laboratory activities in virology focused on the implementation of molecular biology techniques for the diagnosis and characterisation of rotavirus at the national level laboratory.

SURVAC achievements in rotavirus laboratory surveillance from 2009 to 2013

The goal of the laboratory strengthening for the virology component in the SURVAC framework was to increase the capacity of the countries to improve the quality and the range of rotavirus diagnostic testing and establish rotavirus genotyping technique at national level laboratory. The key issues were procurement of equipment, reagent and supplies; training of laboratory personnel; establishment of molecular biology techniques for rotavirus genotyping; management of laboratory data; and enrolment of SURVAC countries laboratories in the WHO PT programme for rotavirus genotyping.

Procurement of equipment, reagent and supplies

Based on the results of laboratory assessment, a list of equipment was established to meet the requirement to set up molecular biology techniques (RT-PCR) and/or EIA technique in SURVAC laboratories. At the beginning of the project, most equipment was provided by WHO/AFRO and reagents by CDC. To build a sustainable system for laboratory items procurement, the WHO/AFRO and WHO country offices managed this procurement since June 2012 and from June 2013, the MoH was supposed to take over the procurement of all laboratory reagents. However, some of the molecular biology reagents used in the laboratories were still provided by either CDC or procured through WHO procurement system with support from WHO country offices.

Training of laboratory personnel

During the SURVAC project implementation, the human workforce was strengthened through technical training and assistance in specialised areas. In all, 23 personnel from the three SURVAC countries were trained in biosafety, quality assurance, QC, specimen handling and transport, rotavirus diagnosis using EIA and genotyping of rotavirus strains using RT-PCR. Two laboratory personnel, one from CAE and one from DRC visited the CDC-Atlanta where they were trained in more advanced molecular biology techniques including sequencing, computer-based sequence analysis and phylogenetic methods for rotavirus genotyping. While at CDC, they also had the opportunity to receive training in measles and rubella genotyping using phylogenetic analyses. Despite a successful implementation of training activities, there still was a challenge of staff retention as the project had to deal with the departure of key personnel involved in the surveillance of rotavirus in CAE and in DRC. This situation, mainly due to the lack of career development opportunities and low salaries, is known to be a major issue in African countries [13]. SURVAC countries are working with partners to address this issue through another component of SURVAC project, the Central Africa Field Epidemiology and Laboratory Training (CAFELT) [14].

Molecular testing capacity for rotavirus genotyping

Prior to SURVAC intervention, none of the laboratories in SURVAC countries performed molecular genotyping testing to support rotavirus surveillance. With support from the African Rotavirus Network [15], stool samples from CAE and DRC were sent with one laboratory member annually to the RRL in South Africa, to perform rotavirus genotyping analyses. Because of the high cost of this process, only few samples (50) were analysed

for rotavirus genotyping by RT-PCR each year [5]. This resulted in few samples being analysed and minimal data on rotavirus strains circulating in these countries. Since the implementation of the SURVAC project, all three countries (3/3) are now performing rotavirus genotyping testing using RT-PCR compared to 0 of 3 countries in 2009 (Table 1). As a result no more samples are sent abroad for rotavirus genotyping. Furthermore, they all participate in external quality control for rotavirus genotyping.

The SURVAC project provided additional resources that helped establish an RT-PCR technique for diagnosis and characterisation of rotavirus in these three countries. Central level laboratories are now carrying out the genotyping of rotavirus samples. This has resulted in an increase in the number of rotavirus samples analysed and strains genotyped per year in each country. In CAE, the number of samples analysed by EIA increased tenfold from 112 in 2007 to 1118 in 2013. The number of strains genotyped increased as well, from 31 in 2007 to 457 in 2013 (Figure 2a). Similar results were observed in DRC, where the number of rotavirus samples analysed by EIA increased five times, from 130 samples in 2009 to 676 in 2013. In parallel, the number of rotavirus strains genotyped increased fourfold, from 39 in 2009 to 181 in 2013 (Figure 2b). However a slight decrease of number of samples genotyped was noticed in 2012, due to reagent stock outs. In CAR, there was no rotavirus surveillance before the implementation of SURVAC project. Data showed that since the establishment of rotavirus surveillance in CAR in 2011, the number of samples analysed by EIA has increased from 54 samples in 2011 to 268 in 2013, and the number of rotavirus strains genotyped from 28 to 58, respectively (Figure 2c).

A total of 2890 samples collected from five sentinel surveillance sites in SURVAC countries, from January 2011 to December 2012, showed that 83 (CAR), 800 (DRC) and 619 (CAE) were EIA positive samples. Of these, RT-PCR-based genotyping testing was performed on 83 (CAR), 400 (DRC) and 569 (CAE). The genotype could be fully identified (G and P types) for 83 (CAR), 272 (DRC) and 545 (CAE) strains, and the rest were partially genotyped. Genotype G2P[6] was most common in CAR (49%) and DRC (10%) while in CAE, G1P[8] (30%) was most common. Detailed information on rotavirus genotypes circulating in SURVAC countries has been reported [9, 16, 17]. Other results found were a decrease in the cost and turnaround time for genotyping analyses; the national laboratories are now generating rotavirus genotype data in their country, instead of once per year in the RRL in South Africa; and a retrospective genotyping of rotavirus samples. Once the genotyping of rotavirus strains was implemented in CAE and DRC, the laboratories were able to go back and genotype more samples collected before the implementation of SURVAC and the results contributed to a better understanding of the diversity and the evolution on rotavirus strains circulating in these countries [9, 16]. In CAR, rotavirus samples collected from a previous pilot study in 2008 were also analysed and genotyped through SURVAC [18].

Management of laboratory data

Laboratory data collection was previously paper-based and upgraded to the computerised system using a standard data collection and reporting tool provided by WHO/AFRO. The computerised system was used to submit monthly reporting of data to the MoH and WHO.

However, some weaknesses were observed with this tool as it did not allow the analyses of rotavirus genotyping information which had to be counted manually. This severely affected and delayed the tracking of genotyping data and therefore the reporting of rotavirus genotyping data.

Epidemiologic and laboratory data produced by the SURVAC project were successfully used by the countries to advocate for the introduction of rotavirus vaccine in their countries. As result, rotavirus vaccine was introduced into the immunisation schedule in CAE in April 2014. DRC and CAR are planning to do so in 2016.

PT program for rotavirus diagnosis by EIA and genotyping by RT-PCR

The SURVAC project has improved QC and quality assurance in laboratory analysis. Since 2012, SURVAC laboratories have been participating in the PT program for rotavirus genotyping provided by WHO and CDC. Table 2 summarises the performance of SURVAC laboratories participating in the PT program. In 2012, the PT score for the EIA testing was 100% for all laboratories and genotyping scores were ranging from 56.3 to 100% in 2012 which was satisfactory performance for a first PT. In 2013, their genotyping score dropped, ranging from 53.1 to 75% for CAE and DRC, respectively. This could have been a result of the logistics and administrative hurdles of WHO/AFRO transferring the responsibility to the MoH for providing the reagents and supplies for rotavirus genotyping as recommended by SURVAC project management committee. In addition to the reagent shortage issue in CAE which prevent laboratory staff from practicing regularly genotyping analysis, the staff turnover certainly affected this proficiency test as personnel responsible for rotavirus genotyping left the laboratory and the staff who performed the test did not have sufficient rotavirus genotyping experience. CAR could not participate in the proficiency testing for genotyping in 2013 due to a political crisis that has affected the country beginning in March 2013. In fact, their PT panel box was opened before receipt in the laboratory and the genotyping primers were missing. In 2014, all SURVAC laboratories passed both EIA and genotyping PT (Table 2).

Lessons learned, challenges and recommendations

The main lessons learned were that with appropriate training, mentorship, human and financial resources, the implementation of molecular technology is feasible in low-income countries, there is a high motivation for capacity development in all laboratories involved in this project, and epidemiology and laboratory teamwork are key for success as this approach enhances data quality and improves surveillance. Most of challenges encountered during the implementation of SURVAC project were already known as they were identified during the assessment.

Shortage of reagents regularly affected laboratory services. The process for obtaining reagents was for the laboratories to send a request to WHO/AFRO which would then procure the reagents and have them shipped to the countries. In practice, the process resulted in receipt only after months of delay and often no receipt some items ordered. The delay was often due to some issue during the clearance of items at the customs or delay in countries using green light for shipments to take place. These challenges are common in some

African countries especially WHO programmes in Central Africa. To build this capacity, the responsibility of reagent ordering was handed over to the MoH in country so that they can manage the orders themselves through local vendors.

Staff turnover was one of challenges faced by SURVAC. Well-trained laboratory staff often moved elsewhere looking for better salaries. The issues of low salaries for laboratory personnel are well known in developing countries. During meetings with health authorities, the SURVAC project have been advocating for the importance of a sufficient budget for laboratory personnel within the MoH budget.

Civil unrest and theft of equipment were among challenges. Theft of laboratory equipment and/or belongings occurred in CAE, DRC and CAR as well. Since the Coup d'etat in CAR in March 2013, laboratory activities have suffered from reagent shortages as the delivery in the country was difficult and safety issues interfered with laboratory supervision. Laboratory personnel from CAR could not attend the training at CDC Atlanta in 2013 because of this civil unrest in their country.

The WHO's electronic database used for collection and reporting of rotavirus surveillance data needed some modifications and improvements. The fact that the database could not analyse rotavirus genotypes has negatively affected the timely reporting of genotype data. The WHO should upgrade the database to improve data analysis and reporting.

To face the challenges related to reagent procurement, we recommend that the MoH update and improve the reagent procurement system in country by allocating some funding to laboratories to allow them to purchase reagents locally. In this case, laboratories should plan to buy some stocks of reagents to avoid reagent shortages that negatively affected laboratory analysis. In regard to staff turnover, we recommend that the MoH improves the career plans for laboratory personnel and plan more funding to hire, train and retain enough laboratory personnel. The African Society for Laboratory Medicine (ASLM) is currently working with African countries to address the issues of career plan for laboratory personnel. It would be good if SURVAC countries could benefits from the help of ASLM. To guarantee the sustainability of this project, we would like to recommend that the countries take ownership of the project. The MoH should use the data from the SURVAC project to advocate for more funding for diseases surveillance, by working closely with the Ministry of Budget and with all external partners.

Conclusion

The SURVAC project has established a strong partnership with participating countries to strengthen disease surveillance and response. The laboratory capacity strengthening component of project has successfully established the national capacity for characterisation of rotavirus strains using robust molecular techniques. This allowed the countries to obtain baseline data for circulating rotavirus strains before the introduction of rotavirus vaccine. Although the focus of the virology aspect of SURVAC was to strengthen the rotavirus surveillance activities, the project has also contributed to strengthen laboratory capacity to detect other vaccine preventable diseases (VPDs) such as measles and rubella. As a

result, laboratory equipment and skills acquired with SURVAC project would enhance the surveillance of other VPDs in the region. This study also showed that to perform good laboratory surveillance it is important that reagents are available in the laboratory at all times and laboratory personnel should be highly trained. These laboratories should continue rotavirus analysis to monitor the impact of the vaccine. Therefore, effort to continue support for laboratory capacity must be pursued by government authorities as recommended by the SURVAC management committee.

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Figure 1.
Geographic location of SURVAC project countries; Cameroon (CAE), Central African Republic (CAR) and Democratic Republic of the Congo (DRC).

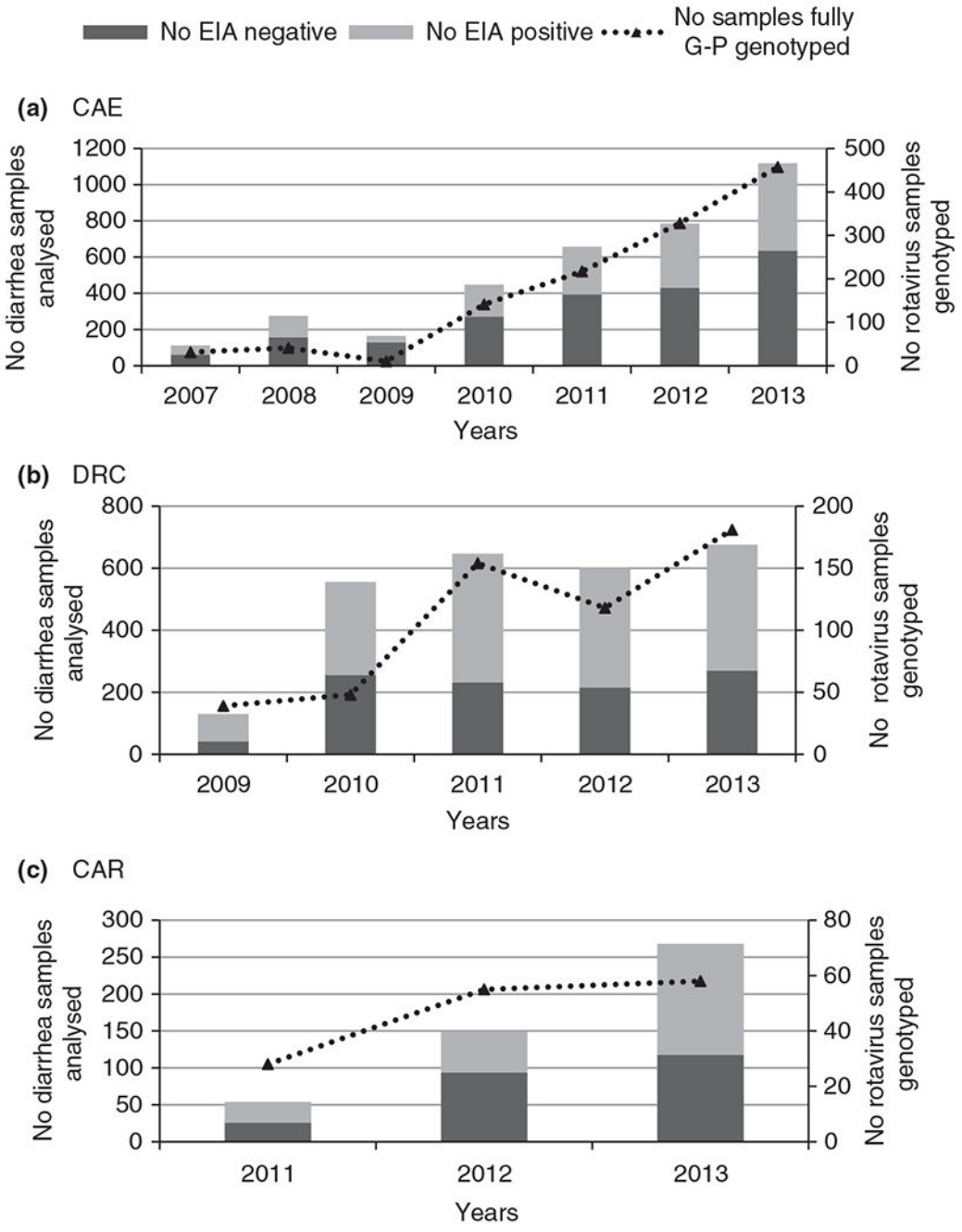

Figure 2.

Annual changes in the number of diarrhoea samples collected and rotavirus strains genotyped from September 2007 to December 2013 in SURVAC countries; (a) CAE (2007–20013), (b) DRC (2009–2013) and (c) CAR (2011–2013).

Table 1

Improvements in laboratory analyses/testing activities after 4 years of SURVAC project intervention

	Number of SURVAC countries performing activities	
Rotavirus-related laboratory activities	**2009**	**2013**
Countries performing rotavirus EIA testing	2/3	3/3
Countries shipping stool samples to RRL for rotavirus genotyping	2/2	0/3
Presence of PCR technical capacity for others VPDs	2/3	3/3
Rotavirus PCR testing in the country	0/3	3/3
Countries participating in PT for rotavirus EIA	2/3	3/3
Countries participating in PT for rotavirus genotyping by RT-PCR	0/3	3/3

Table 2

Summary rotavirus PT panel – EIA and genotyping (RT-PCR) results for SURVAC countries, 2012–2014

	2012		2013		2014	
SURVAC Countries	**EIA (%)**	**RT-PCR (%)**	**EIA (%)**	**RT-PCR (%)**	**EIA (%)**	**RT-PCR (%)**
CAE	100	56.30	100	53.10	90	92.50
CAR	100	100	100	na-conflicts	100	82.50
DRC	100	87.50	100	75	95	85

na-conflicts, non-available due to political unrest in CAR.