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Etiology, epidemiology and clinical characteristics of acute moderate-to-severe diarrhea in children under 5 years of age hospitalized in a referral pediatric hospital in Rabat, Morocco

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Abstract

The objective of the study was to describe the etiology, epidemiology, and clinical characteristics of the principal causes of acute infectious diarrhea requiring hospitalization among children under 5 years of age in Rabat, Morocco. A prospective study was conducted from March 2011 to March 2012, designed to describe the main pathogens causing diarrhea in hospitalized children >2 months and less than 5 years of age. Among the 122 children included in the study, Enteroaggregative *E. coli* (EAEC) and rotavirus were the main etiologic causes of diarrhea detected. Twelve (9.8%) children were referred to the intensive care unit, while 2, presenting infection by EAEC and EAEC plus a *Shigella sonnei* respectively, developed a hemolytic uremic syndrome. Additionally, 6 (4.9%) deaths occurred with EAEC being isolated in four of these cases. Diarrheogenic *E. coli* and rotavirus play a significant role as the two main causes of severe diarrhea while other pathogens such as norovirus or parasites seem to have a minimal contribution. Surveillance and prevention programs to facilitate early recognition and improved management of potentially life-threatening diarrhea-episodes are needed.

Introduction

Diarrheal disease remains a major contributor to illness and death among children less than five years of age in low and middle-income countries, and is also a relevant cause of morbidity among international travelers to these areas (Liu *et al*, 2012). Indeed, pediatric diarrheal disease still accounts for >800,000 deaths per year globally (*circa* 11% of the 7.6 million estimated annual global child deaths) (Lanata *et al*, 2013; Liu *et al*, 2012). Nonetheless, diarrhea-associated mortality is decreasing globally by about 4% yearly; however, the decline in incidence is modest (Levine *et al*, 2012; Liu *et al*, 2012;). It is considered that yearly diarrhea accounts for approximately 2.5 billion cases in children less than five years (WHO/UNICEF, 2009), affecting up to 60% of travelers to some low-income areas (Gascón, 2006; Ruiz *et al*., 2007; WHO/UNICEF, 2009). Additionally, different diarrhea-related severe sequelae have been described, including Guillain-Barré syndrome, hemolytic uremic syndrome (HUS) or reactive arthritis (Fischer Walker *et al*., 2013). In low and middle income countries, in which children may have several episodes/year, diarrhea may lead to nutritional deficits and subsequent growth stunting and decreased cognitive function (Fischer Walker *et al*., 2013). Regarding Morocco in 2009, one study of 2009 (Oudaïna *et al*., 2009) showed that stature-ponderal delay was related to the presence of diarrheogenic parasites in at least 1 of each 6 children. These high levels of disease burden can also be translated into economical costs which affect the healthcare systems and also represent a relevant household economic burden, which is of special relevance in developing areas in which access to inexpensive treatments is difficult (Patil *et al*., 2002; Rheingans *et al*., 2012). These costs are also reflected in social inequities, with a trend towards lower costs related to diarrhea in poorer households, which in some countries may often more frequently affect girls and subsequently result in an increased risk of death (Rheingans *et al*., 2012).

Regarding Morocco, in 2011, 132,000 children less than five years of age were reported to have different degrees of dehydration associated with diarrhea. Of these, circa 23,000 (17.5%) were from the Rabat-Salé-Zemmour-Zair region, especially from urban areas (75.5% of the cases). Additionally, at a national level, 7,247 dysentery cases were reported in 2011, of which 320 were from the Rabat-Salé-Zemmour-Zair region, mostly (315 cases, 98.5%) from urban areas (Ministère de la Sante, 2012).

A series of pathogens, including bacteria, parasites and viruses, may act as the etiological cause of this illness (Mandomando *et al*, 2007, Vargas *et al*, 2004). Nonetheless, the etiological agents of diarrhea vary greatly depending on the geographical origin. In addition, the clinical relevance of each specific pathogen also differs (Kotloff *et al*, 2013; Lanata *et al*, 2013; Pons *et al*, 2014; Prere *et al*, 2006) and, thus, a clear understanding of the prevalent locally-specific etiologies is essential for the design of specific prevention and control measures targeting the main causes.

Although some data on the etiological causes of diarrhea in some Northern Africa countries are available (Al-Gallas *et al*, 2007; Hassine-Zaafrane *et al*, 2011; Hassine-Zaafrane *et al*, 2013; Sdiri-Loulizi *et al*, 2009; Sdiri-Loulizi *et al*, 2011), little is known about the etiology and epidemiology of diarrhea in Morocco. The latest estimates suggest that diarrhea may be responsible for the death of 36 per 1000 live births annually in Morocco (WHO/UNICEF, 2009). However, the few data available regarding the main etiological causes of diarrhea in Morocco are fragmented, and mainly focused on rotavirus, in relation to the introduction of the rotavirus vaccine (Rotarix®) in the year 2010 (Benhafid *et al*, 2012**)**. Data regarding other pathogens are scarce and mostly outdated. The relevance of *Giardia intestinalis* and *Entamoeba histolytica* infections as a cause of diarrhea in this country has also been shown. Thus, a report analyzing 4285 cases of diarrhea showed that these two parasites might altogether account for more than 50% of positive parasite-associated cases (El Guamri *et al*,

2009). Local data about the presence of diarrheogenic bacteria in different food products can also be found (Bennani *et al*, 2011), and specific data regarding infections by *Salmonella* spp. have also been published (Ammari *et al*, 2009**)**. However, a comprehensive description of the epidemiology and etiology of diarrhea in Morocco remains to be performed.

Thus, the main aim of this study was to describe the etiology, epidemiology, and clinical characteristics of the principal causes of acute infectious diarrhea requiring hospitalization among children less than 5 years of age in a referral pediatric hospital in Rabat, Morocco.

Materials and methods

Site description

This prospective study was conducted in the Gastrointestinal Diseases and Emergency Departments at the *Hôpital d'Enfants de Rabat* (HER) in Rabat (Rabat-Salé-Zemmour-Zair region, Morocco). The HER is the only tertiary pediatric hospital in the Rabat-Salé-Zemmour-Zair region but also attends infants from other Moroccan regions (especially from the north of the country). Thus, in 2011 the HER received 120,771 outpatients visits, with 18,471 hospital admissions (Ministère de la Sante, 2012)..

In 2011 the population of the country was reportedly of 32,187,000 inhabitants, 2,872,000 of whom were children (8.9%) under the age of 5 years, and 506,000 children (1.6%) were less than 1 year of age (Ministère de la Sante, 2012), The population of the Rabat-Salé-Zemmour-Zair region was 2,695,000 inhabitants, mostly in the urban area of Rabat (2,270,000 persons, 84.2%), which included 225,000 children under 5 years of age (Ministère de la Sante, 2012).

Study population

The study included children >2 months and less than 5 years of age attending the HER from March 2011 to March 2012, with a primary diagnosis of acute diarrhea, defined as three or more abnormally loose or liquid stools in the previous 24 hours, having begun during the seven days prior to admission to the hospital, with no other known cause of illness, and for whom diarrhea was the principal cause of hospital admission. Diarrhea cases due to chronic ongoing previously diagnosed gastrointestinal diseases were excluded. Likewise, outpatients were not included for not fulfilling the severity inclusion criteria.

Children fulfilling the inclusion criteria and whose parents had signed an informed consent underwent standardized procedures. Demographic, socio-economic and clinical data (including evolution during admission and outcome) were routinely collected following a

125 standardized questionnaire and subsequently double entered using a program written in
126 Filemaker Pro 12 (Filemaker inc., Santa Clara, CA, USA). Treatment of the diarrhea episodes
127 and other related diagnoses was done according to national guidelines and decided by
128 hospital clinicians. Antibiotic therapy was reassessed according to culture results and
129 susceptibility patterns.

130 The rotavirus vaccination status was established either by direct revision of vaccination
131 documents or, in the absence of these documents, by asking the parents / guardians.

132 The study protocol was approved by the Ethics Committees of the Hospital Clinic
133 (Barcelona, Spain) and by the Institutional Review Board (Comité d'Éthique de la recherché
134 Biomédicale) of the Faculty of Medicine in Rabat (Morocco).

135

136 **Case Definitions**

137 All case definitions were based on data obtained at admission from standardized study
138 questionnaires. Fever was defined as an axillary temperature of ≥37.5 ºC, and hyperpyrexia
139 implied a temperature ≥ 39 ºC (Guinovart *et al*, 2008). Nutritional status was based on
140 weight-for-age Z scores (WAZ), calculated using the least mean square method and the 2000
141 CDC Growth Reference (Kuczmarski *et al*, 2002). Dehydration status was established
142 according to standard WHO criteria (WHO, 2005). Dehydration was considered moderate
143 when estimated between 5-10% and severe if >10% (Stoll *et al*, 1982).

144 The minimum community based incidence rates of diarrhea were estimated using the Rabat-
145 Salé-Zemmour-Zair region population as described elsewhere (Kotloff *et al*, 2013).

146

Sample collection:

At enrolment at least 5 ml or 5 gr of stool were collected from each patient by either collection in a waxed cardboard container at the time of defecation or from the diaper if applicable. All samples were processed within a maximum of 12 hours after collection. Additionally, 1 to 2 ml of venous whole blood was collected on admission for biomarker evaluations.

Biomarker determinations:

Procalcitonine (PCT) and C-reactive protein (CRP) levels were determined using miniVIDAS®, (Biomerieux, Marcy-l'Etoile, France) and Microlab 300, respectively.

Bacterial culture

In order to search for the presence of *Shigella* spp., *Salmonella* spp., *Campylobacter* spp., *Vibrio cholerae*, *Yersinia* spp., *Aeromonas* spp. and diarrheogenic *Escherichia coli*, feces were cultured in different media (McConkey, Campylobacter agar, Blood agar, Salmonella Shigella (SS) agar, Xylose Lysine Deoxycholate (XLD) agar, Cefsulodin-Irgasan-Novobiocin (CIN) agar and Thiosulfate-Citrate-Bile Salts-sucrose (TCBS) agar. Bacterial isolates were identified based on growth in the aforementioned media (e.g.: *Salmonella* spp. and *Shigella* spp. were recovered from McConkey, XLD and SS agar; *E. coli* from Mac Conkey agar; *Campylobacter* spp. from Campylobacter agar; while TCBS was used to detect the presence of *Vibrio* spp., CIN to isolate *Yersinia* spp., and Blood agar to isolate *Aeromonas* spp.) and by colony morphology, conventional biochemical techniques (Murray *et al*, 2007) or by an automated system (Phoenix^{TM} 100, Becton Dickinson, Loveton Circle Sparks, USA).

Detection of diarrheogenic *E. coli* strains:

Diarrheogenic strains of *E. coli* (Enteroaggregative *E. coli* - EAEC; Enteropathogenic *E. coli* - EPEC; Enterotoxigenic *E. coli* - ETEC; Diffusely Adherent *E. coli* - DAEC; Enteroinvasive *E.coli* - EIEC; Enterohemorragic *E. coli* - EHEC) were detected by RT multiplex PCR using the primers and methodology described by Guion *et al* (2008).

Parasite identification:

The fecal material obtained from the patients was concentrated using the Ritchie technique, and then stained following the modified Ziehl Neelsen staining procedure in order to detect *Cryptosporidium* spp. (Bailenger, 1973; Tligui & Agoumi, 2006). The presence of *Giardia* spp. and *Entamoeba hystolitica* was determined by microscopy using the Bailenger technique (Bailenger, 1973; Bourée, 1994).

Virus detection:

Nucleic acid for viral studies was extracted using a commercial kit (MagMax^{TM} Total nucleic acid Isolation, Applied Biosystems, Foster City, USA). Detection and genotyping of rotavirus was performed following the procedures by Rodriguez *et al*. (2009). Detection of Sapovirus, Norovirus and Astrovirus was done using the primers described by Yan *et al*. (2003) with a multiplex RT-PCR using the standard conditions described in a commercial kit (Super-script III One step RT-PCR; Invitrogen, Genome Biotechnologies, Casablanca, Morocco). The presence of Hepatitis A was established in a monoplex RT-PCR as previously described (Sanchez *et al*. 2002).

Results

During the 13 month-long study period, 852 out of the 11,799 children (7.3%) attending the Pediatric Emergency Department of the Hôpital *d'Enfants* in Rabat presented with acute gastro-intestinal symptoms, resulting in a minimum community based incidence rate of diarrhea in the region of Rabat-Salé-Zemmour-Zair of 0.35 episodes/100 child-year. Of these, 720 (84.5%) were seen as outpatients and did not require admission, while 132 children fulfilling enrolment criteria and were recruited for the study showing a minimum community based incidence rates of moderate to severe diarrhea in the Rabat-Salé-Zemmour-Zair region of 0.06 episodes/100 child-year. Ten patients were discharged prior to obtaining all the necessary samples, and thus, 122 children were finally included in the analysis (Figure 1).

Diarrhea cases were predominantly seen (73/122; 59.8%) during the cold season (January-March). The mean age of the children recruited was 16.5 months (range 2.4 to 54.2), with a predominance of males (53.3%). Diarrhea episodes had a median duration of 4 days (IQR 1-5), and 103 (84.4%) children presented fever and 108 (88.5%) vomiting. Parents of 29 out of the 122 (23.8%) patients referred pre-admission usage of antibiotics, mainly β-lactam (12 cases) and cotrimoxazole (12 cases). Malnutrition was common among the study population, with over half of the patients recruited (52.4%) showing some degree of malnutrition (WAZ<-1) and almost 15% of the patients being severely malnourished (WAZ< -3). Other relevant clinical and demographic data are presented in Table 1.

A total of 12 (9.8%) children were referred to the intensive care unit (ICU), while 2, presenting EAEC and EAEC plus a *Shigella sonnei* infection, respectively, developed a HUS. Six out of these 12 children (50%) died, representing 4.9% of the total number of children recruited. In four out of these six cases, EAEC infection (one coexisting with an astrovirus) was identified. The final diagnosis obtained by the study clinicians after review of the whole hospitalization file in cases who died corresponded to acute gastroenteritis/diarrhea (4 cases),

219 acute renal failure (one patient with prolonged hospitalization of 14 days) and disseminated
220 intravascular coagulation (DIC, one case). Importantly, neurological abnormalities
221 (convulsions and/or impaired consciousness) were of note during these diarrhea episodes
222 ending in death (table 2).

223 Regarding specific infection biomarkers, 57.0% and 29.0% of the patients with available
224 results (n=100) presented increased levels of PCT or CRP, respectively. The mean PCT value
225 was significantly higher in patients with bacterial infection (18.0) compared to the mean
226 value of patients with viral infection (2.0; $p<0.001$). However, the mean CRP value was
227 comparable in both bacterial and viral infections, being below the threshold defined as
228 elevated in both cases (0.05g l^{-1}). Interestingly, patients in whom neither viruses nor bacteria
229 were isolated from stools, showed the highest CRP and PCT levels (Table 3).

230 At least one pathogen was isolated in 89 out of the 122 fecal samples (73.0%). The most
231 frequent etiological agents were diarrheogenic *E. coli* (71 isolates, 58.2%), rotavirus (21,
232 17.2%,) belonging to genotypes G1P8: 16 (76.2%); G3P9: 4 (19.0 %) and G8P9: 1(4.8%);
233 and *Shigella* spp. (8, 6.5%) (Table 4). Co-infections were frequent and present in 25 (20.5%)
234 of the patients, including rotavirus and *E. coli* (EAEC) (7 cases, 28%), and rotavirus and *E.*
235 *coli* (DAEC) (3 cases, 12%) as the most frequent combinations, while three or more
236 pathogens were recovered in another two patients (Table 5).

237 The most frequent diarrheogenic *E. coli*, included EAEC (47 cases; 38.5%), followed by
238 DAEC (15; 12.3%), EPEC (7; 5.7%), ETEC (2; 1.6%), and two (1.6%) isolates presented
239 both the EAEC and DAEC characteristics. Neither EHEC nor EIEC were isolated.

240 Thirty-eight out of the 122 children (31.1%) had received at least one dose of the currently
241 implemented rotavirus vaccine (Rotarix®). Six had received three doses, while 17 had
242 received two doses and 15 reported to have received only one dose of the vaccine. The
243 remaining 84 children recruited were not vaccinated or vaccination data was not documented.

Rotaviruses in feces were mainly recovered (14 cases, 66.7 %) from children apparently not vaccinated or for whom data were unavailable. However, rotavirus infections were also detected in children with partial or complete rotavirus vaccination: 2 cases in patients having received 1 dose; 5 further cases in children having received 2 doses; and 1 case in a child reporting three doses (Table 6). Finally, rotavirus infections seemed to show a clear seasonality, being mostly detected during the cold season (Figure 2).

Discussion

Diarrhea remains a relevant cause of childhood morbidity and mortality in Morocco, as previously suggested by the scarce reports available from this country or from the Maghreb area (Bourrous *et al*., 2010; INSPA, 2005). Indeed, while diarrhea-related admissions were relatively uncommon in this hospital (only 122 cases during a 13-month period), mortality associated with this syndrome in Rabat was high (6 deaths, 4.9%), especially when compared to the recent results of a large multicenter study on the global etiology of diarrhea showing a varying range of diarrhea-attributable case fatality rates (from 0.13% in India to 7.5 in Mozambique, with a mean of 2%) (Kotloff *et al*, 2013). In four out of these six deaths, diarrheogenic *E. coli* (3 EAEC, 1 DAEC) was detected in feces. Despite attributing causality to these microbiological findings, the determination of the precise cause of death in these patients is challenging and may be inappropriate without adequate post-mortem confirmation, and without thorough exclusion of other potential co-morbidities. On the other hand the role of diarrheogenic *E. coli* as a cause of child mortality has been robustly documented and reported elsewhere (Kotloff *et al*, 2013; Lanata *et al*, 2013; Nataro *et al*. 1998).

Two cases of HUS were detected as severe complications among children admitted with acute diarrhea. HUS is a severe complication which is often associated with the presence of specific pathogens such as EHEC or *Shigella* spp. (Khan *et al*, 2013; Fischer Walker *et al*, 2012). In our series no EHEC isolate was found, and in both HUS cases an EAEC isolate was detected, one being associated with *Shigella sonnei* co-infection. Although *Shigella dysenteriae* type 1 is, by far, the member of the *Shigella* genus most often implicated in the development of HUS (Fischer Walker *et al*, 2012), a recent report from Bangladesh confirmed the potential of *S. sonnei* as an etiologic trigger for HUS (Khan *et al*, 2013). Despite the recent outbreak in Germany involving EHEC/EAEC isolates (Aurass *et al*., 2011), to our knowledge, the role of EAEC as a cause of HUS remains undescribed, and thus,

no direct association may be extrapolated from current data. Despite data regarding HUS in middle and low-income countries being scarce, this syndrome is the most relevant cause of acute kidney injury among pediatric populations, especially affecting young children (Hofer *et al*., 2014). Regarding Morocco, HUS has been described as the second cause of acute infantile renal failure (Bourquia *et al*., 2002) and, similar to other diarrhea complications, this syndrome contributes to long-term diarrhea-related morbidity. Thus, in some cases HUS may lead to the development of different chronic problems such as long-term hypertension, diabetes mellitus or neurological sequelae among other adverse extrarenal outcomes, as well as different degrees of long-term impairment in renal function. Indeed, around 5% of cases develop chronic renal failure requiring dialysis or kidney transplantation (Spinale *et al*. 2013), which in low income countries may lead to death due to the lack of adequate treatment.

As anticipated, and in accordance with previous studies having shown the potential of PCT as a predictor of bacterial blood infections (Diez-Padrisa *et al*, 2012), PCT levels were significantly higher amongst bacteria-related diarrhea cases compared to virus-related diarrheal episodes. The higher levels of PCT observed in diarrhea cases in which no specific pathogen was isolated suggest the presence of unidentified bacteria / parasites more than the presence of viruses.

A low number of parasitic infections has been described in studies of etiology of diarrhea in the Maghreb area (Al-Gallas *et al*, 2007). This low prevalence was confirmed in our series, in which only two parasitic infections (*G. intestinalis, E. histolytica*) were detected, being lower than that observed in a previous study performed in the same hospital with identical methodologies, in which a total of 10 *Giardia intestinalis* isolates were detected in a series of 63 children (15.9%) with stature-ponderal delay (Oudaïna *et al*. 2009). A possible explanation for the difference in positivity between the 2 studies may be that diarrhea

associated with parasites does not require hospitalization, and no parasites were detected in the present series. However, it is likely that the use of molecular techniques for parasite detection would result in a higher detection capacity.

The vast majority of the cases of diarrhea described in this report were related to bacterial infections, predominantly caused by diarrheogenic *E. coli*, particularly due to the EAEC and DAEC pathotypes, but also by *Shigella* spp., *Salmonella* spp. and *Campylobacter* spp. The role of EAEC as a relevant cause of pediatric diarrhea has been described worldwide (Kotloff *et al*, 2013; Mandomando *et al*, 2007; Ochoa *et al*. 2009; Vargas *et al*., 2004). Interestingly, the second most frequent diarheogenic pathotype of *E. coli* isolated was neither EPEC nor ETEC similar to previous studies (Mandomando *et al*, 2007; Vargas *et al*., 2004), but rather DAEC. Two *E. coli* isolates presenting mixed characteristics (mixed EAEC/DAEC pathotypes) were detected. The presence of diarrheogenic isolates presenting mixed characteristics of two different pathotypes is not a new finding (Aurass *et al*., 2011; Ruiz *et al*., 2008). Furthermore, the presence of EAEC/DAEC has recently been described in South America (Garcia *et al*, 2011). This fact is of special concern because it might reflect either the intercontinental spread of new mixed pathotypes, or their parallel evolution in geographically distant areas. The public health risk of such mixed pathotypes was clearly established in the recent Germany EHEC/EAEC outbreak which resulted in approximately 4000 infected persons including more than 900 cases of HUS and 59 deaths (Karch *et al*., 2012).

Rotavirus followed by astrovirus accounted for the majority of viral-related diarrheal episodes. Rotavirus infections were essentially recovered during the coldest months, as described elsewhere (Benhafid *et al*. 2013), even in the same geographical area (Hassine-Zaafrane *et al*, 2011). Rotavirus was the most frequent virus involved in the development of cases of diarrhea, and ranking as the specific second cause of diarrhea after EAEC isolates. Three different genotypes were detected: G1P8, G3P9 and G8P8. While G3P9 and G8P8 are

not included in the recently introduced rotavirus vaccine in Morocco, G1P8, the most prevalent genotype detected, is adequately covered by this vaccine (Benhafid *et al*. 2013). The G1P8 genotype was detected in 4 children partially or fully vaccinated. This might be explained by the fact that a low incidence of new cases would be expected in children adequately vaccinated.

Although the relevant role of rotavirus and the proportion of cases attributable to astrovirus are in agreement with what has been previously described in the north of Africa (Sdiri-Loulizi *et al*, 2009), the low incidence of norovirus is in clear disagreement with previous data in the Maghreb area (Hassine-Zaafrane *et al*, 2013). Thus, no clear explanation is available to explain the lack of norovirus as a cause of pediatric diarrhea in our series. On the other hand, sapovirus has been described as a common cause of mild-to-moderate diarrhea, usually not requiring hospitalization, in Tunisian children (Sdiri-Loulizi *et al*, 2011) which may explain why so few cases of sapovirus were detected among our series of moderate-to-severe patients with diarrhea requiring admission.

To the best of our knowledge, this is the first report providing a comprehensive scenario of the etiological causes of severe pediatric diarrhea in Morocco. Despite some limitations such as the inability to detect some recently described emerging pathogens such as *Campylobacter concisus* (Nielsen *et* al., 2013), this study sets the basis for further research regarding pediatric diarrhea in the area, and advocates for the establishment of adequate hospital-based microbiologic surveillance systems. The low-to-moderate burden of diarrhea-related admissions among Moroccan children, as detected in the HER of Rabat poses, however, a major public health problem, particularly due to the unexpectedly high associated case fatality rates. We have demonstrated the relevant role of diarrheogenic *E. coli* and rotavirus as the two main causes of severe diarrhea in this area, with the lower contribution of other pathogens such as norovirus or parasites being of note. These data call for the implementation

of better surveillance and prevention programs, as well as improvement in the early recognition and management of potentially life-threatening episodes of diarrhea.

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525

526 **Legend to Figures**

527

528 Figure 1: Study profile

529 Figure 2: Rotavirus detection in patients with acute diarrhea according to seasonality (period

530 March 2011-March 2012)

531

Table 1: Clinical and demographic data of patients included in the study

Age (months)	
• Mean (SD)	16.5 (11.5)
• Range	2.4-54.2
Sex	
• Male	65/122 (53.3%)
• Female	57/122 (46.7%)
Number of referred stools/day	
• Mean	6.1
• range	3-15
Duration of diarrhea episode	
• Median (IQR)†	4 (2-5)
Blood in feces	3/122 (2.5%)
Mucus in feces	79/102 (77.5%)
Fever	103/122 (84.4%)
Complications	
• Transfer to Intensive care unit	12/122 (9.8%)
• Hemolytic uremic syndrome	2/122 (1.6%)
• Death	6/122 (4.9%)
Vomiting	108/122 (88.5%)
• Mean number of episodes	5.1
• Duration (Days): Median (IQR)	3 (2-5)
Breast feeding/feeding difficulty	95/122 (77.9%)
Paleness	62/103 (60.2%)
Dehydration	121/122 (99.8%)
• Mild	51/121 (42.1%)
• Moderate	64/121 (52.9%)
• Severe	6/121 (5.0)
Nutritional status	
• WAZ score (Mean; SD)	-1.1 (1.8)
• Malnutrition (WAZ<-1)‡	54/103 (52.4%)
Hemoglobin (g/dL)	
• Mean	11.4
• Range	6.4-14.8

† IQR: Interquartile range; ‡ WAZ: Weight-for-Age Z score

Table 2 Clinical descriptions of severe cases of diarrhea resulting in death

Patient	Age (months)	Medical insurance	Days with diarrhea	N*	Convulsions	dehydration	ATB†	Creatinine	ICU‡	Days of hospitalization	HUS§	Microorganism	Final diagnosis
DR0007	23.4	No	3	10	Yes	Severe	No	NA#	Yes	2	NO	EAEC/DAEC	Acute gastroenteritis
DR0009	21. 7	No	3	4	Yes	Moderate	No	0.37	Yes	2	NO	None	Acute gastroenteritis
DR0013	4.4	No	4	NA	Yes	Moderate	No	0.7	Yes	0	NO	Astrovirus /EAEC	Acute gastroenteritis
DR0016	3.0	No	3	5	No	Moderate	No	2.94	No	14	NO	EAEC	Renal failure
DR0020	24.9	No	1	6	No	NA	Yes	1.21	Yes	0	NO	EAEC	DIC¶
DR0054	8	No	5	4	Yes	Severe	No	NA	No	1	NO	NA	Acute gastroenteritis

* N: Number of stools/24h;
†ATB: Pre-admission antibiotic intake;
‡ ICU: Intensive Care Unit;
§ HUS: hemolytic-uremic syndrome;
# NA: Not available;
¶ DIC: Disseminated Intravascular Coagulation.

Table 3: Laboratory findings according to microorganisms detected in stools

	Bacteria	Virus	Mixed Bacteria/virus	No pathogen detected
Procalcitonin (ng/ml)				
• Mean	18	1.98	18.7	42.49
• Range	0.05-243.7	0.06 -16.18	0-370	0-300
C Reactive Protein (mg/dl)				
• Mean	2.5	2.14	2.3	5.0
• Range	0.1-19.2	0.1-7.7	0-19.2	0-35.9

Table 4: Microorganisms detected

Microorganisms	Number of cases (%)
Bacteria	
EAEC	47 (38.5)
DAEC	15 (12.3)
EPEC	7 (5.7)
ETEC	2 (1.6)
EAEC/DAEC	2 (1.6)
Shigella spp.	9 (7.4)
Salmonella spp.	5 (4.1)
Campylobacter spp.	5 (4.1)
Protozoa	
Giardia intestinalis	1 (0.8)
Entamoeba histolytica	1 (0.8)
Virus	
Rotavirus	21 (17.2)
Astrovirus	6 (4.9)
Hepatitis A	1 (0.8)
Norovirus	1 (0.8)

EAEC: Enteroaggregative *E. coli*
DAEC: Diffusely adherent *E. coli*
EPEC: Enteropathogenic *E. coli*
ETEC: Enterotoxigenic *E. coli*
EAEC/DAEC: Microorganisms presenting EAEC and DAEC mixed characteristics

Table 5: Common co-infections in patients with acute diarrhea

Coinfection	cases
Rotavirus - *E. coli* (EAEC)	7
Rotavirus - *E. coli* (DAEC)	3
E. coli (EAEC)-*Shigella*	3
E. coli (EAEC)-*Campylobacter*	3
E. coli (EAEC)-Salmonella	2
Astrovirus - *E .coli* (EAEC)	2
Rotavirus- *E. coli* (EPEC)	2
Astrovirus-*E.coli (*DAEC*)*	1
Norovirus- *E. coli* (EAEC)	1
E.coli (DAEC)-*Campylobacter*	1
Hepatitis A- *E. coli* (EPEC)	1
Rotavirus-*E. coli* (EAEC)- *Campylobacter*	1
Rotavirus-*E. coli* (DAEC) - *Salmonella*	1

EAEC: Enteroaggregative *E. coli*
DAEC: Diffusely adherent *E. coli*
EPEC: Enteropathogenic *E. coli*
ETEC: Enterotoxigenic *E. coli*

Table 6: Rotavirus genotypes and vaccination status of rotavirus-associated diarrheal episodes

	0 doses	1 dose	2 or 3 doses	Rotarix®
G1P8	12	1	3	*
G3P9	1	0	3	●
G8P9	1	0	0	●

*Serotypes covered by Rotarix® ●Serotypes not covered by Rotarix®

Figure 1

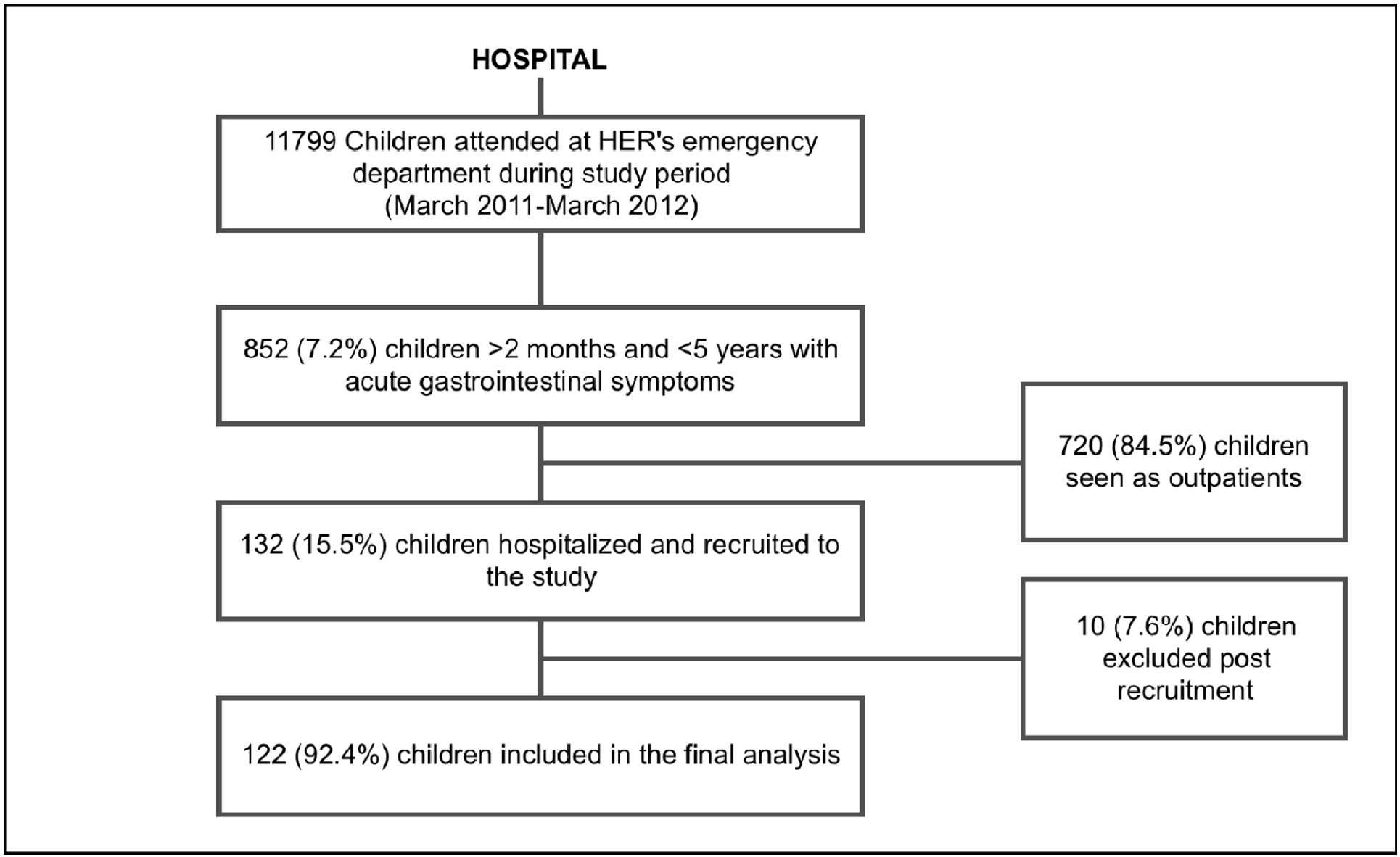
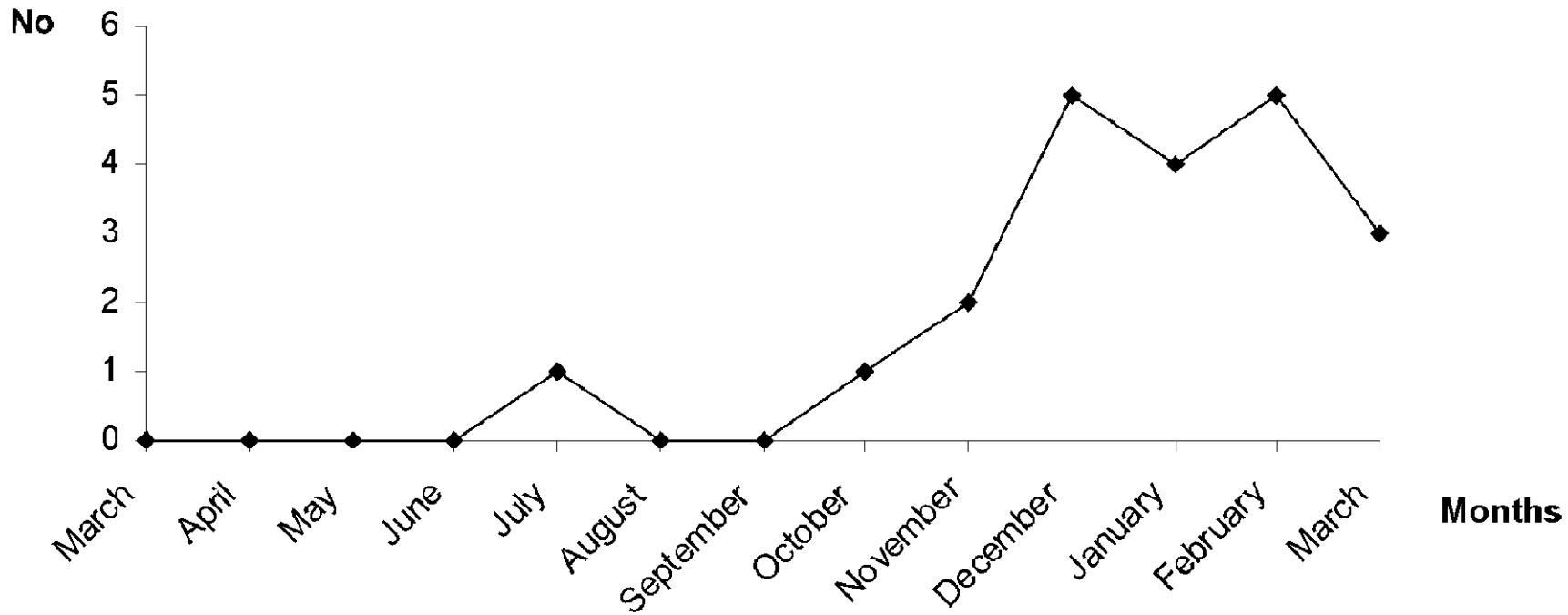

HOSPITAL
11799 Children attended at HER's emergency department during study period (March 2011-March 2012)
852 (7.2%) children >2 months and <5 years with acute gastrointestinal symptoms
720 (84.5%) children seen as outpatients
132 (15.5%) children hospitalized and recruited to the study
10 (7.6%) children excluded post recruitment
122 (92.4%) children included in the final analysis

Figure 2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