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Research Article

Gastro-Enteritis

Overlooked Etiologies of Childhood (0-59 Months) Gastro-Enteritis in a Semi Urban Community

René Fotsing Kwetche P.[1*], Gamwo Dongmo S.[2], Ngaha A.[3], Kaba K.[4], Pulchérie Tamatcho Kweyang B.[5], Kaptué L.[6]

DOI:

[1*] Pierre René Fotsing Kwetche, Institut Supérieur des Sciences de la Santé de Bangangté, Université des Montagnes, Bangangté, Cameroon.

[2] Sandrine Gamwo Dongmo, Cliniques Universitaires des montagnes, Bangangté, Cameroon.

[3] Augustin Ngaha, Cliniques Universitaires des montagnes, Bangangté, Cameroon.

[4] Kourouma Kaba, Université des Montagnes, Bangangté, Cameroon.

[5] Blandine Pulchérie Tamatcho Kweyang, Faculty of Science, University of Yaoundé I, Yaoundé, Cameroon.

[6] Lazare Kaptué, Faculty of Science, Université des Montagnes, Bangangté, Cameroon.

The present study aimed at investigating protozoan, bacterial, fungal and viral (rotavirus, adenovirus) etiologies of diarrhea amongst children aged 0 through 59 months and identifying a few factors likely to influence prevalence rates of this disorder in Bangangté-Cameroon. A questionnaire was designed to gather pieces of information on life style during specimen collection. Microbial screening was conducted on stools specimens according to standard protocols for all microbes. Out of total of 200 stool samples subjected to laboratory examination 64 % were elucidated. In decreasing frequencies, outstanding etiologies included enteroagregative E. coli (EAEC, 39.4%), Candida albicans and Klebsiella pneumonia (15.5% each), Ascaris lumbricoides (8.1%), rotavirus and adenovirus (5.4% and 4.5%, respectively). Screening for EAEC and Klebseilla is not a usual exercise because their role in diarrhea was ignored so far setting. For most of these pathogens, detection rates were associated with age of candidate. Natural protection appeared to increase rapidly against C. albicans and rotavirus. It was also observed that a large number of parents (66.7%) do normally not report their children cases to health facility. When they were appropriately informed, however, number of reported cases could be ten times higher, consistent with crucial role of education in preventing childhood diarrhea.

Keywords: Childhood diarrhea, Klebsiella, EAEC, Viruses

Corresponding Author

Pierre René Fotsing Kwetche, , , Institut Supérieur des Sciences de la Santé de Bangangté, Université des Montagnes, Bangangté, , Cameroon.
Email: prfotsing@udesmontagnes.org

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Introduction

Consistently throughout years many reports have recognized childhood gastro-intestinal disorders (GIDs) as major health concerns in developed and developing nations worldwide. They represent in all settings a high burden for both the general population welfare and their economy (Walker et al., 2013; Bhutta et al., 2013; Gill et al., 2013). Diarrhea is one of the three most common causes of death amongst children under five, with the highest prevalence rates in developing countries (Farag et al., 2012; Mondal et al., 2012; Walker et al., 2013; Bhutta et al., 2013; Chopra et al., 2013). Paradoxically, it hardly deserves (if ever) the appropriate medical attention in a large number of these heavily burdened settings. Ranges of factors can influence de severity of cases from one geographic location to the other. This may include the causative agent involved, the purchasing power, access to general education, climate, availability and affordability of primary healthcare facilities, human resources for health, nutritional status and believes (Walker et al., 2012; Gastanaduy et al., 2013; Kotloff et al., 2013; Malek et al., 2006). These factors may act in a synergetic manner within a community.

Generally limited to viruses in developed nations, etiologies of diarrhea also include infectious agents like bacteria, microscopic fungi and protozoa in resource-limited areas (Cairn Cross et al., 2010). Morbidity and mortality due to communicable diseases in general have greatly declined in industrialized nations with the improved living standard (Nguendo Yongsi, 2011). In developing countries, the backbone to controlling infectious diseases (IDs) would basically rely on detecting and/or identifying these infectious agents and designing appropriate prevention and case management strategies, a paramount challenge for local health authorities. But the limited facilities for research do not allow achievement of the intended objectives in many cases. Further, survey initiatives in developing countries are concentrated in metropolis while remote populations are those in which the above conducive factors most likely combine. In Bangangté, this challenge is far to be addressed. In this semi-urban community a mixed population develops spontaneously, favored by the presence of higher schools that attract individuals from different living standards and educational Background. The population growth contrasts with the shortage of basic commodities expected to accompany urbanization like drinking water supply and human resources for health. As all sustainable control strategies should take into account database-dependent context knowledge, we carried out the present survey to gather useful information on childhood GIDs in Bangangté. More specifically, our intent was to identify bacterial, fungal, viral (rotavirus and adenovirus) and protozoan etiologies, and a few environmental factors that are likely to influence the prevalence rates of infectious childhood diarrhea in the study area. This study was conducted in agreement with the general policy of the "Université des Montagnes" about the welfare of local populations.

Materials and Methods

Study population, participants and specimen collection

This study was conducted in Bangangté, a semi-urban areas characterized by a cosmopolite population consisted of endogenous farmers, students attending higher institutions of learning and small traders. The general population was sensitized through banderols and mass media on the necessity to take all children who frequently released loose, liquid or bloody excreta/stool (with or without other signs of discomfort like fever and vomiting), to the hospital for free biological examination and eventual medication in case of microbial infection. A questionnaire was also prepared to collect general information about the knowledge of the meaning and significance of diarrhea in a child, the means of prevention and management of cases, the age of the participant, and the availability of drinking water, among other. The institutional ethical comity approved the research protocol under the authorization number 2013/073, before it was undertaken.

After inform consent was obtained from the parents or guardian, stool specimens were collected from children aged 0 to 59 months and transferred into two lidded tubes, one containing 9 ‰ sodium chloride water and the other 10% formalin for microbiological screening. Once collected, the specimens were immediately conveyed to the Laboratory of Microbiology of the school hospital, "the Cliniques Universitaires des Montagnes" (Banékané) for microbial analysis.

Microbiological analysis

1. Parasitological screening: This was conducted by microscopy using direct examination; first, between slide and cover slide with physiologic water and iodine. In parallel, a loop-full (≈15-20 g) of the specimen was transferred in a test tube containing a mixture of formalin and ether (10/3 v/v proportion). The set was centrifuged 5000 rpm for 3 minutes. Upon completion of centrifugation, the supernatant was removed and discarded while the concentrate (at the tube bottom) was collected and process as earlier (with 9 ‰ saline water and iodine). All preparations were observed at 10x and 40x for the presence of parasites, repeated twice and the microscopic screening conducted by two professionals separately.

2. Bacteriological and fungal screening: Culture and identification were conducted according to the Repository in Medical Microbiology (Rémic, 2007). Briefly, a suspension was prepared by homogenizing 15-20 g (4mL if liquid) of the stool sample in 10 mL of 9 ‰ sterile saline. Agar media in Petri dishes (Hektoen, MacConkey and Sabouraud agar, Liofilchem®) were lawn using approximately 10 µL from the specimen suspension. In a parallel manner, 15-20g (4mL if liquid) of the stools sample was dispensed in 13 mL of Muller Kauffmann Tetrathionate Broth (MKB), Liofilchem®. All preparations were allowed to incubate aerobically overnight (Hektoen, MacConkey plates, at 37°C and Sabouraud at 30°C). The MKB preparation was re-sawn on Hektoen for additional 18-24 hrs incubation at 37°C. Upon completion of incubation, suspected colonies were isolated for morphological and biochemical identification. Susceptibility tests were then performed on pathogenic bacterial and fungal strains. The testing and test interpretation were done as recommended by the «Comité de l'Antibiogramme de la Société Française de Microbiologie» (CA-SFM-2012). Test for aggregative E. coli was conducted as previously (Fotsing Kwetche et al., 2008) and by surface biofilm formation in Muller Hinton Broth (Nataro and Kapper, 1998).

3. Viral detection: Detection of viruses was based on that of viral-coat-borne proteins, the highly immunogenic structural capsid-borne Viral Protein 6 (VP6) for rotavirus, and Hexon for adenovirus. The lateral flow chromatographic immunoassay using the Rota-Adeno (catalog N° 751120-20) strip using A combination of monoclonal antibodies and latex particles on a solid phase was used in this essay.

The assay was conducted as recommended by the manufacturer. Briefly 1mL of the sample diluents was dispensed in a plastic test tube. To this about 2-5 g or 100 µL (if liquid) of the stool sample was added and adequately emulsified in the buffer, then centrifuged 5000 rpm for 1 minute. With a Pasteur pipette, 500 µL of the resulting supernatant was collected and dispensed into another test tube. The specimen area of the strip was then immersed in this suspension and the set allowed to incubation 5 to 10 minutes at room temperature. Test reading and interpretation relied upon an internal test control for the validity of the procedure. Development of additional colored bands on the test area indicated a positive test, enabling detection of the targeted viral determinants separately.

Results

Data analysis revealed that 66.7% of parents were not interested by modern medicine in case of diarrhea. After communication prior to the study, case report was 10-fold higher compared to previous year in the same hospital (monthly average 69 against 7). A total of 206 diarrheal cases were observed. Out of these, 200 stool samples were collected and submitted to microbial screening. Five parents did not accept to participate while one child died before the specimen was collected. Data recorded provided pieces of information used to summarize the prevalence rates distribution as illustrated in figure 1.

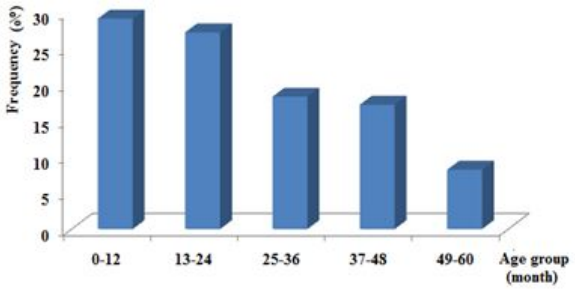

Figure 1: Prevalence rate distribution

Overall, the prevalence was age-dependent. Vulnerability also decreased gradually with increased age of the participants (56.3% between 0 and 24 months; and 8.2% between 49 and 59 months).

The macroscopic feature (physical appearance) of the specimens prior to microbial analysis revealed four categories: watery, loose, mucus-rich without blood, and bloody mucus-rich excreta. Upon complete screening, 226 organisms were identified from 128 cases (64% of elucidated cases). Some etiologies were more associated with the stool physical appearance than the others (Table 1).

Table 1: Distribution of etiologies according to excreta physical feature

Etiology group	Macroscopic feature of specimen and isolation frequency							
	Watery	%	Loose	%	NBMR	%	BMR	%
Viruses								
Rotavirus A	12	18.47	0	0	0	0	0	0 50
Adenovirus	5	7.69	0	0	3	4.29	2	
Bacteria								
K. pneumoniae	7	10.69	7	8.04	19	27.14	2	50
Salmonella spp	5	7.69	2	2.30	4	5.71	0	0
ECEA*	7	10.69	58	66.67	24	34.28	0	0
Shigella sonnei	2	3.1	0	0	0	0	0	0
Protozoa								
A .lumbricoides	5	7.69	12	13.79	2	2.86	0	0
G.intestinalis	2	3.10	0	0	0	0	0	0
E. histolytica	0	0	3	3.45	2	2.86	0	0
T. trichiura	2	3.10	0	0	0	0	0	0
Cyst of S.hominis	2	3.10	0	0	0	0	0	0
H.diminuta	2	3.10	0	0	0	0	0	0
Microscopic fungus								
C. albicans	14	21.58	5	5.75	16	22.86	0	0
TOTAL	65	100	87	100	70	100	4	100

*: Enteroagregative E. coli; NBMR: Non bloody mucus-rich; BMR: Bloody mucus-rich

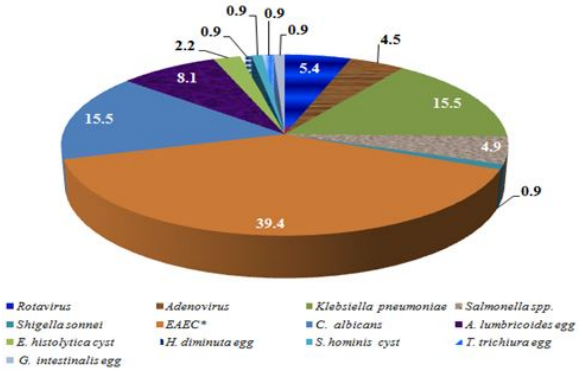

Figure 2: Frequency (%) of etiologic agents

Obviously, the most frequent etiologies of watery cases were bacteria (32.17%)

And viruses (26.16%). Bacteria were most frequently isolated from the non-bloody mucus-rich specimens (67.14%), primarily EAEC, which was also most commonly recovered from other categories than the bloody mucus-rich samples.

Overall, microbial etiologies could be classified into 13 groups. Regardless of the age of the participants, estimates of microbial isolation rates (Figure 2) highlighted the overwhelming frequency of three, representing 70.4% of all identifications. They included EAEC, C. albicans and K. pneumoniae. Ascaris lumbricoides and Rotavirus A followed in decreasing frequencies.

With regard to the age, this distribution followed the pattern displayed in table 2.

Table 2: Etiologies isolation rates (%) per age group

Etiology	Age group (months) /(%)				
	≤ 12	13 -24	25 - 36	37 - 48	49 – 59
Rotavirus A	83	17	0	0	0
Adenovirus	20	70	10	0	0
Klebsiella pneumoniae	25.7	34.3	0	25.7	14.3
Salmonella spp.	27.28	18.18	18.18	0	36.36
Shigella sonnei	0	100	0	0	0
ECEA*	33.33	21.34	21.34	21.34	2.65
C. albicans	51.5	25.7	20	2.8	0
A. lumbricoïdes	0	36.84	26.33	21.05	15.78
E. histolytica	0	0	20	40	40
H. diminuta	0	0	100	0	0
S. hominis	0.0	50	0.0	0.0	50
T. trichiura	0.0	0.0	100	0.0	0.0
G. intestinalis	0.0	100	0.0	0.0	0.0

*: Enteroagregative E. coli

Etiologic types displayed an age-dependent distribution pattern like the prevalence (except bacteria frequent in all age groups). Rotavirus A and C. albicans overwhelmed other organisms in children bellow 12th; Adenovirus was highest between 13th and 24th (highest polymorphism, ≈70%, in this age group) while protozoan infections dominated from the 13th months and above. Most common association were bacteria-protozoa and bacteria viruses (encompassing 70.6% of co-infection cases).

Investigation into likely conducive factors indicated that source of water was most obvious. About 62% of families are not connected to formal distribution

Networks and rely on water from doubtful origin (stream, wells and others). In addition, close to 95.5% of connected families also use resources like streams and wells.

Discussion

The present study revealed that infectious diarrhea cases are caused by a wide range of microorganisms. In the study area, about 66.7 percent of parents do no seek modern medical attention in case of diarrhea, while close to 75 % of those coming to the local health facility believe that diarrheagenic children who take water would rather exacerbate the course of the disorder (with excess fluid in the gut). This is major evidence that failure to report is associated with lack of strategies expected to be developed by all stakeholders in the locality. Otherwise, quality health care service is associated with population involvement. In fact, the indigenous populations who are yet to accommodate modern lifestyles most likely misuses available facilities like water closets, as tap water is not often supplied. Previous studies (Fotsing Kwetche et al., 2008; Guendo Yomsi, 2011) reported an association between diarrhea under five years of age and poor water management in the household.

Overall, microbial agents of diarrhea were greatly diversified (13 microbial types recovered). Most important were bacteria, the least expected of which were strains of Klebsiella pneumonia (not investigated in the routine process). Members of the genus Klebsiella are invasive bacteria (and hosts of wet environments), known for their involvement in a range of infections in the gastrointestinal and urinary tracts, pneumonia and other bodily systems (Brisse and Verhoef, 2001; Phuong et al., 2003; Yingchun et al., 2014). Strains of Klebsiella were isolated (alone or in association) from about 15.5% of specimens, like C. albicans. Ignoring its role in diarrhea likely precludes elucidation of many cases in clinical laboratories in Cameroon (less than 30% of cases are elucidated in the best equipped). Infection by strains of this bacterial species may be followed by the internalization of enterocytes by mutualists like E. coli or residential protozoa in the gastro-intestinal tract. In such cases, adequate caretaking would require specific drug therapy (antibacterial and anti-parasitic drugs, for instance). Usually, however, such possibilities

Are ignored and only one or the other is considered at once, contributing to a prolong course of disease and the resulting morbidity. It is, therefore, not a surprise that IDs caused by strains of this bacterial species become public health concern. Many authors have emphatically regarded pneumonia and diarrhea due to K. pneumoniae as the most important causes of morbidity and deaths among immune depressed adults and children under five (Phuong et al., 2003; Al-Salihi et al., 2012; Bhutta et al., 2013; Gill et al., 2013). New investigations are required to clarify and confirm these findings and the role of these organisms in pneumonia and other airway disorders in association with childhood diarrhea in the study area. Research orientation in this field is crucial to master case management, evaluate the burden of Klebsiella-associated IDs, and design convenient and sustainable context-based strategies for disease control and prevention.

EAEC was reported by previous authors as one of the major causes of persistent diarrhea in children in other settings (Germani et al., 1998; Okeke et al., 2000; Gassama et al., 2001; Fotsing Kwetche et al., 2008). Data analysis revealed that isolation of strains from this pathotype was common (39.4%) in the present survey. Rarely investigated or regarded as non significant in most cases, colonization by EAEC results in malnutrition and low Z-score. Like many other etiologies, contamination by strains of this E. coli pathotype is associated with low hygiene standard.

Viral etiologies investigated included Rotavirus and Adenovirus. If Rotavirus A was reported in Bangangté in a previous survey (De Donno et al., 2012), specimens are screened for Adenovirus in the area for the first time. These viral etiologies were shown to be typically associated with watery stool specimens. Evidences are provided on the fact that genetic variability is high in rotavirus and that strains distribution is dependent upon geographical area (De Donno et al., 2012; Anochie et al., 2013). Immunochromatography results revealed a Rotavirus A prevalence rate of 5.4% in the present work, lower than that reported by De Donno et al. (7.4%). Both studies were conducted during the rainy season, but in the current one, more candidates were recruited. In addition, the samples were collected during the heavy downpour of August-September rather than June-August when rains are not so frequent.

Seasonality is known as a major determinant guiding the distribution and dissemination of rotavirus, the highest rates of infection occurring during the dry season (Armah et al., 2010; Anochie et al., 2013). Up to recent dates in Cameroon, studies were concentrated on parasitic infections and classical bacterial agents of gastroenteritis like Salmonella, Shigella and enteropathogenic E. coli (EPEC) (Fotsing Kwetche et al., 2008; Guendo Yomsi, 2011). Based on most recent works (Ngun Ndze et al., 2012; De Donno et al., 2012; Nordgren et al., 2012; Anochie et al., 2013; Bonkoungou et al., 2013) and the present study, the necessity of inserting viruses on the list of pathogens targeted in the routine process in clinical laboratories is highlighted.

Findings from the present study revealed that most affected children were in the age groups less than 23 months, consistent with Anochie et al. (2013); and can be explained by the fact that immunity develops as children grow older. Similar explanation can be given for C. albicans in which the isolation rates dropped very rapidly with increased age. Some earlier reports observed that maternal protection against rotavirus is highest in newborns and infants during the first three months of life and decreases with increasing age (Parashar et al., 1998; Parashar et al., 2003; Parashar et al., 2006). For these authors, the most vulnerable age groups were 24 months and above. Still others (Nordgren et al., 2012) observed that some strains could induce more severe symptoms than the other, however, implying that severity may not only be age-dependent but strain strain-dependent as well.

Rotavirus (RV) and Klebsiella infections can be prevented through immunization (Bhutta et al., 2013; Gill et al., 2013). The recent introduction of the active vaccine, Rotarix®, in Cameroon will theoretically prevent about 33.4% of diarrheal infections caused by RV with regard to circulating strains (De Donno et al., 2012), but cross-reacting antigens among RV strains may make this percentage much more larger (Glass et al., 2012; Gastanaduy et al., 2013).

More studies are required to justify the necessity to undertake immunization against Klebsiella pneumoniae associated diarrhea and pneumonia (another heavy public health burden) in the same age groups as discussed by Rudan et al. (2007).

Amazingly, protozoan infections remain high (≈14.8%) within the study population. In fact, large spectrum anti-parasitic drugs are available and affordable in the setting. Free drug distribution is also frequently organized by the local health authorities in primary schools. It may therefore, not be understood why infection rates due to this organisms remain so high. At least, three options can be anticipated, however: first, all children do not take the medicine (for one reason or the other); second, the dose administered is not adequate (not all the required doses are administered to the children); third, the children are permanently in contact with sources of contamination (relatives who have not received the drug in the household, for instance). The third alternative, most likely, is also known to ensure maintenance and dissemination of other etiologies of infectious diseases. The low isolation rates of Salmonella spp., and Shigella spp., could not allow a clear understanding of their roles in childhood diarrhea. The prevalence rates recorded were closer to those usually obtained in the few laboratories where they are targeted. Only a larger scale research may tell more about the issue.

Conclusion

This was the most comprehensive study so far conducted in childhood diarrhea in Bangangté. A wide range of microorganism was identified. Screening for EAEC and *Klebsiella* as etiologies of diarrhea is not a usual exercise in clinical laboratories in the setting.

Factors like shortage of water and multiplicity of water resources could not orient the sources of contamination. There still a lot to do in order to ensure proper use of the available human and material resources. The role of hygiene is paramount in preventing IDs regardless of the etiology incriminated. The necessity of inserting viruses, EAEC and Klebsiella on the list of gastro-intestinal pathogens targeted in the routine process in this age group is also highlighted.

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