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Molecular Epidemiology of Rota Virus in Acute Diarrhea in under Five Children in Lubumbashi, Democratic Republic of Congo

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Abstract: ***Background: Rotavirus infection is responsible of 20 to 70% hospital admissions and between 6000,000 to 2 million of deaths per year in developing countries. A number of circulating genotypes are different from one geographical zone to another compromising the possibility of having a vaccine with equal efficacy. Thus, characterizing circulating genotypes is a critical step to assist in making a vaccine with proven efficacy against all circulating rotavirus genotypes. Methods: Between 2009 and 2015, children admitted in Jason Sendwe Hospital were included in the study and stool specimen were collected and analysed by PCR. Molecular characteristics of rotavirus strains were assessed. Results: In total, 483 children were included in the study and their stool specimens were analyzed. 219 of them (45.3%) were positive for rotavirus. The median age of positive patients was seven months (Q1:4.5-Q3:10). Female patients represented 52.5% (115/219). Genotypes were analyzed for 70 specimen out of the 219 (32%). Predominant genotypes found were G1P [8] (18.6% or 13/70) and G2P[6] (18.6% or 13/70) followed by G1P[6] (8.6% or 6/70) and G3P[6] (8.6% or 6/70). Conclusion: The results of this study could be used for the selection of the vaccine to be used and the evaluation of its impact in Lubumbashi.***

Keywords: Diarrhea, Rotavirus, Children, Lubumbashi

1. Introduction

Acute diarrhea remains one of the major public health issues in a number of developing countries. According to the World Health Organization (WHO), rotavirus contributes to around 40% of hospitalizations and the main cause of severe diarrhea in under five children worldwide [1].

According to WHO in 2013, worldwide, nearly 215,000 (19,000 to 233,000) deaths of under five children were attributed to gastroenteritis due to rotavirus (GERV) compared to 528,000 (465,000 to 591,000) in 2000. Around 90% of deaths due to rotavirus occurred in low income countries in Africa and Asia where there was an association with poor quality of health care. Four countries (India, Nigeria, Pakistan and the Democratic Republic of Congo [DRC]) contributed to nearly the half (49%) of all deaths in under five children in 2013 [1,2]. National DRC estimations indicated that deaths due to rotavirus in under five children in 2013 were 13,526 (range between 12,534 to 14,517). This represents around 4.2% of all the deaths in under five children and the mortality rate per specific cause (death attributed to rotavirus in under five children by 100,000 children) was 113.6 [2].

Vaccination against rotavirus modifies the epidemiological profile of GERV, reduces the number of consultations and induces indirect protection. In fact, there is an evidence of a reduction incidence of GERV in the age group of children who have had vaccination [3].

In 2009, WHO recommended all countries to include vaccination against rotavirus in the routine expended immunization program [1].

Recognition of the rotavirus as a major cause of diarrhea in children has led to extensive research for the interventions to reduce the incidence of this disease, characterize the circulating genotypes and develop an effective vaccine to protect against all the genotypes of rotavirus [4].

Rotavirus is a double-stranded RNA virus of the family Reoviridae. The virus is composed of three concentric shells that enclose 11 gene segments. The outermost shell contains 6 structural proteins (VP) and 5 or 6 non structural proteins [5]. In human, at least 12 different antigens VP7 (G serotypes) and 15 antigens VP4 (P serotypes). As serotypes G and P can be combined independently, a binomial typing system is used to identify the strains [5].

Actaully, in the vast regions of the world, 5 combinations are responsible of around 90% of all human rotavirus infections: G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8]. G1P[8] is the most prevalent. On the other hand, data from Asia and Africa reveal a greater diversity of types of circulating rotavirus. The prevalent serotypes may vary from one season to

another, even within the same geographic zone. There is not usually correlation between serotype and the severity of the disease [5,6].

Several genotypes are in circulation and are different from one geographic area to another, jeopardizing the possibility of having an effective vaccine everywhere. Thus, characterization of circulating genotypes is necessary for the development of an effective vaccine against all the genotypes of rotavirus. New emerging rotavirus genotypes have been isolated from across Africa in recent decades [7]. For example, strains G8 in South Africa, Ghana, Nigeria, Malawi, Tunisia and Madagascar [7-11], strains G5 in Cameroon [12] and strains G9 in Ghana and Guinea-Bissau [7,13].

To date, only a limited number of studies on circulating rotavirus genotypes were published for the Democratic Republic of Congo (DRC) [14-17] but no such studies have conducted in the former Katanga Province and particularly in Lubumbashi.

The present study describes the epidemiology and molecular characterization of rotavirus strains linked to acute gastro-enteritis in under five children admitted for acute diarrhea at the Jason Sendwe Provincial Hospital of Lubumbashi in DRC between 2009 and 2015.

2. Material and Methods

A prospective and descriptive study was conducted from the 1st January 2009 to the 31st December 2015 in the pediatric department of the Jason Sendwe General Provincial Referral Hospital in Lubumbashi, DRC.

DRC is located is part of the Central Africa with a tropical climate with two seasons: hot season and rains from September to April and cold and dry season from May to August. Lubumbashi is the second biggest town of the DRC with an estimated population of three millions. Jason Sendwe Hospital is a referral government hospital that deserves the vast majority of people of the Haut-Katanga Province and other surrounding Provinces.

Stool specimen were collected in under five years children admitted for acute diarrhea. Information about the socio-demographic parameters and clinical symptoms of patients were collected on the printed survey form.

Samples were tested using immuno-enzymatic ELISA (IDEIA™ Rotavirus; DakoCytomation). Positive samples were sent to the DRC National Institute of Biomedical Research based in Kinshasa and at the Medical Research Council Diarrheal Pathogens Research Unit, University of Limpopo (Pretoria, South Africa) for the complete detection and molecular characterisation of rotavirus strains.

Rotavirus RNA was extracted from stool samples using phenol and chloroform. It was precipitated with ethanol and the RNA segments were separated by electrophoresis on polyacrylamide gel followed by a staining with silver nitrate [18]. Then RNA was extracted using the Trizol™ method. Serotypes G and genotypes P were determined by methods of amplification and sequencing based on the reverse transcription- polymerase chain reaction (RT - PCR) [19].

The analysis was perfomerd on the incidence of GERV and the general characteristics (age and sex), clinical and molecular strains of rotavirus in the study population.

The study protocol received the clearance of the Medical Ethical Committee of the University of Lubumbashi and approval from the Medical Director of the Sendwe hospital. All specimen were collected from inpatients for therapeutic reason or diagnosis. Specimen were collected by taking into account different communes of Lubumbashi, the availability of the laboratory technician and the sampling equipment. Sampling was not exhaustive.

There was not discrimination on considering patients based on the test results. There was no names recorded into the database and analysis was performed anonymously.

3. Results

483 stool specimen were collected and analyzed from under five children admitted and treated for acute diarrhea from 2009 to 2015 in the pediatric department of the Jason Sendwe Hospital of Lubumbashi. 219 were tested positive for rotavirus giving a prevalence rate of 45.3%. The median age of positive patients was seven months (Q1:4.5- Q3:10) and 90% of them had less than one year. Female patients represented 52.5% (115/219). The main symptoms associated with diarhea were fever (90.2%) and vomiting (77%).

Among the 219 tested positive, 32% (70) had the genotypes analyzed by RT-PCR. For the G serotype, the predominant genotype was G1 (32.86%) followed by the G2 strains (22.86%) and G3 (8.57%). Mixt infections were seen. There were association between G1 and G2 in 1.43% of all cases. For the P serotypes, predominant genotypes were P[6] (50%) and P[8] (24.29%) (Table 1). Three mixt infections were found: P[6,8] (4.29%), P[4,8] (2.86%) and P[4,6] (1.43%).
In brief, the principal strains of rotavirus detected were G1P[8] (18.6% or 13/70) and G2P[6] (18.6% or 13/70) followed by G1P[6] (8.6%) and G3P[6] (8.6%). We also noted that 15.71% of samples did not have G or P serotypes.

Table 1: Rotavirus genotypes in under five children admitted for acute diarrhea at the Jason Sendwe hospital of Lubumbashi, DRC

Genotype	G1	G2	G3	G12	G1,12	No G-type	Total
P[6]	7	13	6	4	1	4	35 (50.00%)
P[8]	13	1	0	1	0	2	17 (24.29%)
P[6,8]	2	1	0	0	0	0	3 (4.29%)
P[4,8]	0	0	0	0	0	2	2 (2.86%)
P[4]	0	1	0	0	0	0	1 (1.43%)

P[4,6]	1	0	0	0	0	0	1 (1.43%)
No P-type	0	0	0	0	0	11	11 (15.71%)
Total	23 (32.86%)	16 (22.86%)	6 (8.57%)	5 (7.14%)	1 (1.43%)	19 (27.14%)	70 (100%)

4. Discussion

We report detection and characterization of rotavirus strains in episodes of acute diarrhea in children under five years in Lubumbashi from 2009 to 2015.

Stool specimen were collected from 483 children throughout the year without taking into account the season. Rotavirus was detected in 43.5% of samples collected. This proportion is lower than what was seen in other studies conducted in Kinshasa (DRC) (61 and 70%) [16,17] and in Ouagadougou (Burkina Faso) (64.2%) [20]. These authors explained that the high proportion could be attributed to the fact that specimen were collected during the cold and dry season where there is an expected spike of rotavirus infection. In Kisangani (DRC), a prevalence rate of 29% from inpatients was reported between 2007 and 2010 [15].

We also found that rotavirus infection mostly affected under one year children. This is similar with other African studies [17,20,21].

Our results indicate that GERV were mainly caused by G1P8, G2P6 and G3P6 strains. The G1P6 strain was frequently found in Africa [7,11] and the P6 genotype that was predominant in studies conducted in the DRC [15,17] was also found in Nigeria [22,23]. This provides evidence of extreme heterogeneity in genotypes strains identified throughout Africa.

In addition, our results indicate that 18.6% of specimen consisted of the strain G1P8. In developed countries, this strain represents around 70% of incidence while in Africa the incidence is lower than 23% [7, 17, 24].

We noticed a high proportion of P6 genotype (50%) with a high number of unusual strains (G12P6, G1P6, G3P6). Heylen et al. [15] found that P6 genotype represented 52% in a study conducted in Kisangani (DRC). These unusual combinations found in our study and in other studies conducted in DRC could represent a potential link between human and animal rotaviruses due to zoonotic infections.

11 of our specimen (15.71%) did not have G or P serotypes. This may suggest the presence of unusual zoonotic genotypes. This observation was also reported in a recent study in Kampala (Uganda) by Bwogi et al. [25]. These authors reported that there was an increase of the risk of rotavirus infection between the consumption of raw vegetables and having dogs in the family [25]. We may assess this association in a further study. These results reinforce the hypothesis that the big diversity in rotavirus strains in developing countries could be linked through a contact between human and animals [15]. Although the rotavirus transmission is mainly due from one person to another, it is not excluded that a transmission between animals and humans occur and may play an important role. Leaving in close contact with pigs and using animal feces in agriculture for vegetables may constitute a corridor of transmission. This way of transmission is compatible with the high diversity in observed constrains in developing countries and the high proportion of co-infection with multiple rotavirus strains.

The vast majority of different genotypes G and P and the predominance of P6 detected in our study (50%) challenge the efficacy of the existing vaccine against rotavirus. A vaccine that includes different genotypes identified is recommended to prevent morbidity and mortality attributed to GERV in our settings.

5. Conclusion

In the study conducted in hospital in Lubumbashi, 45% of acute gastro-enteritis in under five children are due to rotavirus infection. A number of rotavirus genotypes were detected. These results provide an overview of circulating rotavirus strains in Lubumbashi. They will assist public health authorities in defining an appropriate and adequate vaccination strategy against rotavirus infection.

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7. Authors' Contributions

MSK, OM, AMM, DNM and ONL carried out the conceptualization, design, data collection and analysis for the study. MSK, OM, PL, ONL, JPVG and SOW contributed to the interpretation of the findings and the drafting of the article. Both authors read and approved the final manuscript.

8. Conflicts of Interest

The authors declare no conflict of interest.

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