

Circulation of Enteric Viruses in Tirana, Albania: Situation Ten Years After the First Study

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SUMMARY

A variety of enteric bacteria, viruses and protozoa are the leading causes of morbidity and mortality worldwide. To understand the evolution of gastroenteritis in Albania, in terms of distribution of aetiological pathogens, a one-year observational study was repeated in 2017, ten years after the first study performed in 2007.

The data still show a clear circulation of viruses that cause gastroenteritis. Compared to the previous study in 2007, the data from the 2017 study showed the incidence of Norovirus and Adenovirus were significantly higher (p value <0.05), while Rotavirus was verified at a similar incidence rate.

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Diarrhoeal diseases are the leading cause of mortality and morbidity worldwide, killing 3.3 to 4.0 million people per year, particularly in developing countries where the diseases also have a heavy economic impact (World Health Organization, 2020). In 2019, diarrhoeal diseases were globally placed as third among the top ten causes of DALYs (Disability Adjusted Life Years) in children younger than 10 years, while in developing countries they still represent the second principal cause of fatality in children under the age of 5, and it is estimated that they are responsible for the deaths of about 525,000 children every year (GBD - Global Burden Disease - 2019 Diseases and Injuries Collaborators, 2020). Food and water contaminated by human faeces from municipal sewers, septic tanks and latrines are the most frequent vehicles in the transmission of etiopathogenic microorganisms causing diarrhoea (Lanata, 2003). It is now also well known that various bi-directional associations exist between oral health and gastro-intestinal diseases (Contaldo *et al.*, 2021). Diarrhoea is only a symptom of an infection caused by bacterial, viral and parasitic microorganisms and can spread as direct transmission from person to person due to

poor personal hygiene and/or an overcrowded family environment (Bandsma *et al.*, 2019). Viral infections in particular are the second most common cause of diarrhoeal disease and, in industrialized countries, show broad circulation compared to those caused by bacteria and parasites (Greenwood *et al.*, 2011). Other causes, such as septic bacterial infections, are likely to be responsible for an increasing proportion of all deaths associated with diarrhoea, malnutrition, or compromised immune systems, which are considered risk factors for developing clinical, diarrhoea potentially lethal, (Walker *et al.*, 2013). Diarrhoea is more frequently caused by gastrointestinal infections, of which rotavirus has been recognized as the main cause of severe acute diarrhoea-inducing gastroenteritis (Dennehy *et al.*, 2012). But even if the incidence rate of rotavirus infections in developing countries is not very different from that in industrialized countries, the death rate is reported as significantly higher (Hallowell *et al.*, 2020). Therefore, in order to understand the cost-benefit ratio of diarrhoea-preventing vaccines, the cost of direct medical expenses in vaccinated people (\$0.72) versus unvaccinated individuals (\$40.32) was calculated (Takala *et al.*, 1998) (Ahmeti *et al.*, 2015).

Regarding Albania, systematic analysis conducted by the Global Burden Disease study during the period 1990-2019 calculated the specific weight of two major causes of infant mortality: lower respiratory infections (LRI) and diarrhoea. Both diseases were significantly reduced during this period: from 75.4 to 27.9/100,000 for LRI and from 4.3 to 0.3/100,000 for

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diarrhoea, respectively (GBD 2019 Diseases and Injuries Collaborators, 2020). On the other hand, morbidity from infectious diseases is still high, representing 11% of the total disease burden. In 2010, diarrhoea alone had an annual incidence of 72/100,000 inhabitants (crude rates, all ages), which can still negatively affect children's health status (Burazeri *et al.*, 2014). The present study methodology was submitted and approval was received from the National Committee of Bioethics, before the samples were collected and data were analysed (October 2013 - December 2014). In the present survey, clinical and epidemiological data from a sample of 167 cases of gastroenteritis were filled out by the staff of Paediatric Health Service. During a one-year period, the study recruited a sample of 167 children aged 1 month to 7 years, of both sexes, hospitalized at Paediatric Service of the University Hospital of Tirana (Albania). The study criteria were: paediatric age interval, hospitalization in infectious paediatric wards, and presence of diarrhoea. The data resulting from the epidemiological analysis were subsequently compared with those reported in a 2007 paper to establish any significant differences in the circulation of enteric viruses in the paediatric population of the city of Tirana, Albania and its surroundings after almost 10 years (Fabiana *et al.*, 2007).

A specific questionnaire, aiming to collect individual data from each child affected by diarrhoea, was carefully filled out by the staff of Paediatric Health Service. The questionnaire reported useful information for describing general socio-economic and demographic conditions, as well as the children's health status (cases of overcrowding in the household, etc.), lifestyle and hygiene in the child's home (presence of toilet or latrine, etc.), the type of foods and method of alimentation, the accessibility to drinking water from the domestic network or the type of water used for drinking, the use of mineral water for infants and other more specific questions regarding gastroenteritis.

A total of 167 human stool samples from children suffering from diarrheal diseases were collected in faecal collectors (V302-F; Starplex Scientific, In., Ontario, Canada) and prepared in 10% w/v in complete buffered salts (PBS). After 10 minutes of vigorous mixing, samples were centrifuged at 4500 rpm/15 min and the supernatant collected, divided into aliquots and frozen at -80°C until their transport, via routine specimen transfers, to the University of Rome "Tor Vergata" (Italy) for virological testing. All samples were accompanied by requisition forms documenting the timing of specimen collection.

All of the stool samples were tested by RT-PCR for Astrovirus, Rotavirus, Norovirus GI and GII, Adenovirus (<http://www.ncbi.nlm.nih.gov/BLAST>). To avoid a different test sensitivity, the molecular test performed in 2007 and described in a previous paper concerning the influence of enteric viruses in Albania

were scrupulously repeated together with the same lab procedures and techniques (Fabiana *et al.*, 2007). All of the enzymes for the molecular test were obtained from Promega, Milan, Italy.

Genomic viral RNA was extracted using the TRizolLS reagent (Gibco, BRL, Milan, Italy) and the final pellet was resuspended in 55-60ul of sterile water. Genomic viral DNA was extracted using the Seek Viral DNA kit (Talent, Milan, Italy). The final pellet was resuspended in 60ul of pure ribonuclease-free water and divided into aliquots. The amplified product was purified using the QIAgen PCR purification Kit (QIAgen, Milan, Italy) and the reading was performed using a New Generation of Sequencing. The obtained sequences were analysed at the NIH site (www.ncbi.nlm.nih.gov/BLAST).

Current data suggest an evident circulation of enteric viruses with little or no significant difference from previous results obtained almost 10 years ago (Fabiana *et al.*, 2007).

According to the results, 125 out of 167 (74.8%) stool samples were positive for one virus whereas 13 out of 167 (8.3%) for two or more viruses. From the total of 132 samples that were positive to at least one virus, Rotavirus was the most representative with 93 positive samples (70%), Astrovirus 1 (0.8%), Norovirus GG2 4 (3%), Adenovirus 32 (25.7%). From the family of Adenoviruses, Adenovirus 40 and Adenovirus 41 were identified in 2 and in 10 samples, respectively. The negative samples represent 20.9% of the total (Fabiana *et al.*, 2007).

Two subsequent studies, 10 years apart, measured the yearly frequency of diarrhoea commonly caused by viruses in children hospitalized with the diagnosis of gastroenteritis. *Table 1* shows the two samples, composed of 313 and 167 children, studied in 2007 and 2017, respectively, and the relative number of children in whom each virus was identified. To determine which of these viruses have statistically changed their distribution in the paediatric age group in Albania, we can run a z test comparing two proportions in the two samples.

According to the statistical test, the Rotavirus and Astrovirus were distributed similarly in the two different studies, since the z value is equal to 0.2 and 1.5, respectively, and the relative p values are both higher than 0.05 (0.8 and 0.1). The opposite is true for the other two viruses: the Norovirus increased, while the Adenovirus decreased from 2007 to 2017. According to the statistical test, z values are 3.0 for Norovirus and 2.9 for Adenovirus. In both cases, the relative p value is much lower than the threshold value of 0.05

In the total of positive samples, 41.3% belong to females and 58.7% to males; 56.7% of children live in the urban area while 43.3% reside in the suburban and rural areas around Tirana (*Table 2*).

The age distribution of the samples is 28.1% 1-6

Table 1 - Differences in virus circulating in year 2007 vs year 2017.

Year	Rotavirus		Astrovirus		Norovirus		Adenovirus		Neg. samples		Tot Sample
	No	%	No	%	No	%	No	%	No	%	
2007	105	71,4	5	3,4	19	12,9	18	12.3	166	53.1	313
2017	93	70	1	1	4	3	34	26	35	21.1	167
p-value	> 0.05		> 0.05		< 0.05		p value < 0.05)		n/a	n/a	n/a

Table 2 - Data obtained from the questionnaires.

Period	Gender		Mean Age	Residence Area	
	Female	Male	Months	Urban	Rural
2017	41.3	58.7	15.8 (2-84)	56.7	43.3
2007	42.8	57.2	17.6 (2-144)	48.2	45.7
NUMBER OF ADULTS LIVING IN THE HOUSEHOLD					
	2 - 3		4 - 5	6 or more	
2017	15.2		49.4	35.4	
2007	17.8		44.5	37.7	
NUMBER OF CHILDREN LIVING IN THE HOUSEHOLD					
	1	2	3	≥ 4	
2017	39.7	36.4	13.3	10.6	
2007	33.2	33.2	18.2	15.4	
DOMESTIC AND DRINKING RUNNING WATER			TYPE OF DOMESTIC HYGIENIC SERVICE		
	Yes	No	Latrine	Toilet	
2017	39.7	36.4	32.3	68.4	
2007	33.2	33.2	43.0	97.0	

months, 30.1% 7-12 months, 18.3% 13-18 months, 15.7% 19-24 months, and 7.8% more than 24 months. The mean age is 15.6 months with a range of 2-84 months (Table 2).

Another variable relates to the number of persons living in the household, where 15.2% of patients live with 2-3 adults, 49.4% live with 4-5 adults, and 35.4% with six or more adults. With regard to the presence of children in the household, 39.7% live with one other child, 36.4% with two other children, 13.3% with three other children, and 10.6% with four or more children (Table 2).

Only 39.7% of the interviewees declared they had running water 24 hrs/day, while 36.4% have no running water at home (Table 2). Depending on the availability of water, one third (32.3%) have just latrines while two thirds (68.4%) have a toilet at home.

Water is the principal vehicle of the enteric pathogen and the amount and quality of water is extremely important (Gorski *et al.*, 2019). Upon analysing the individual data of both surveys, no significant differences can be noted; thus, in the city of Tirana and its surroundings the social-economic situation, lifestyle and hygiene in the home appear practically unchanged in the last 10 years.

The official statistics of the Tirana University Hospital covering the same period as the study samples were randomly collected. The three separate services of the paediatrics department, where gastroenteric cases were normally admitted, reported around 1,000 cases, corresponding to more than 3,500 recov-

ery days for the period from January to November 2016. To these figures should be added the children hospitalized in other regional and district-based hospitals, as well as outpatients accessing day hospitals or hospital-based polyclinics for paediatric services in the entire country.

In conclusion, the virological analyses carried out after about 10 years in the city of Tirana and other surrounding cities continue to confirm the substantial presence of circulating enteric viruses.

If we consider the absence of a wastewater treatment plant, the picture of massive environmental pollution appears correct and consistent with what was declared in all the results.

Although there is still a large circulation of viruses, the number of gastroenteritis cases and the number of hospitalizations have decreased, indicating a clear epidemiological transition with a significant shift from an infectious disease to non-communicable diseases (Stroni *et al.*, 2014). In the current condition, considering the important contribution of Rotaviruses in the overall rate of diarrheal disease in the paediatric population of the city of Tirana, Albania and its surroundings, the specific vaccine (Takala *et al.*, 1998) would certainly be an important contribution, able to prevent at least two thirds of the burden of cases of diarrhoea in all clinical forms, both clinical and morbidity, and cases of hospitalization and deaths related to Rotaviruses, which continue to heavily affect the country's paediatric population.

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