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Estimation of Rotavirus Associated Diarrheal Disease Burden Amongst Primary School Children of Sindh

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ABSTRACT

Introduction: Human Rotavirus Virus (HRV) is amongst the common enteric viral diarrheal diseases that indirectly or directly influence school-going children in low-income countries. Inadequate information exists on the presence of rotavirus, with reference to drinking water supplies of primary schools in Sindh, Pakistan.

Aims & Objectives: We estimated the risk of rotavirus-associated diarrhea through drinking water. The study further compared the HRV-associated risk of diarrheal disease by a type of water source.

Place and Duration of Study: A cross-sectional survey was conducted in ten representative districts of Sindh from February 2022 to December 2022. The samples for drinking water were collected from primary schools of ten representative districts of Sindh. This study was a part of the WASH Project funded by USAID (USPCASW Seed Grant P-II)

Material & Methods: We selected 425 samples of drinking water from primary schools based on pre-defined selection criteria. We used a Quantitative Microbial Risk Analysis using indicator organisms, i.e., *E. coli,* to predict the possible health risks of rotavirus. Data was entered and analyzed on SPSS version 26. The graphs were developed using Arc GIS version 3. Morbidity and mortality were predicted using the Quantitative Microbial Risk Assessment (QAMRA) model.

Results: Our data revealed that the highest daily risk of HRV-associated diarrhea amongst school children was 11 per 10,000 schoolchildren, resulting in 8.4% annual risk, and the minimum risk was estimated to be 1 in 10,000 children. The burden of diseases for rotavirus using the QAMRA model revealed the severity of the diarrhea. Majority of the children presented with mild diarrhea (86%) followed by severe, and the probability of death was less than <1%. The daily risk of HRV infection was highest (estimated to be 7 and 11%) in pupils of Southern Sindh, with an annual risk of 17.4% to 40%.

Conclusion: Our study concluded that the children in the primary schools of Sindh were exposed to poor drinking water quality. The surface water source poses the highest risk of HRV-related diarrhea to school children. Thus, it is highly recommended that point-of-use drinking water treatment systems be adopted. The water sanitation and hygiene (WASH) resources are interlinked, so each resource impacts the other; hence, schools urgently need to invest in providing adequate WASH facilities to stop enteric virus transmission through drinking water sources.

Keywords: Diarrheal risk, Human Rotavirus infection, Primary school children, Waterborne Diseases

INTRODUCTION

Water is a typical reservoir for rotaviruses. Viruses enter the drinking water through anthropogenic causes such as leaking sewage, agriculture runoff, poor septic system wastewater discharges, and urban runoff. Moreover, in many areas of Sindh, wastewater sources are used to irrigate vegetables[1,2]. The enteric viruses enter the environment via the biosphere along with the fecal-oral route by either fomites or direct human contact[2,3]. These viruses contain unique characteristics of causing illness by transferring from person to person with minimal viral doses, i.e., <20 particles[4]. Human rotaviruses (HRV) are food- and water-borne enteric viruses that significantly increase the risk of acute viral hepatitis and non-bacterial gastroenteritis, both of which can be fatal[5]. These viruses spread to other prospective hosts directly or indirectly by reproducing in the gastrointestinal tract of the infected individual and releasing large amounts into the stool[5]. Amongst viruses, the genre HRV is considered one of the leading causes of diarrhea in children and infants. Based on their genetic and antigenic properties, the HRV contains seven species from A to G. However,

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worldwide HRV accounted for 90% of gastroenteritis[6]. The oral rotavirus vaccine has been launched in many countries as a primary preventive measure to curtail the enormous burden of gastroenteritis cases linked to HRV. Sadly, the vaccine has not yet been incorporated into numerous developing nations' routine immunization programs[7].

The majority of HRV cases are documented in places where the water is contaminated, and there is poor sanitation, overcrowding, and poor hygiene. Additionally, 114 million cases of HRV, including both symptomatic and asymptomatic patients, are documented annually[8,9]. Four nations—India, Nigeria, Pakistan, and the Democratic Republic of the Congo—accounted for over half (49%) of all Rota-related deaths in children under the age of five worldwide[10].

A study in Pakistan attributed 40% of all deaths and 30% of diseases to unsafe water. In contrast, HRV and other waterborne diseases are reported as the leading causes of death[11]. These disease outbreaks can be very serious and significantly impact the general populace's social, economic, and health conditions. People may require months to recuperate from illnesses and get back to their normal lives, schools, or jobs. Poor WASH services constitute a significant barrier in Pakistan against tackling human enteric viral diseases, mainly including HAV and HRV[11].

When compared to family settings, schools, especially primary schools, have paid less attention to water, sanitation, and hygiene (WASH). Therefore, given that kids spend a lot of time at school, school WASH is crucial. Second, due to unsanitary settings, the high amounts of person-to-person interaction exposes young kids to numerous infections, including HRV. Thirdly, low immunity and under nutrition are related. These increase the risk of illness brought on by these enteric viruses[14]. Finally, the morbidities related to enteric viruses negatively impact pupils' performance. Hence, the study group is vulnerable to serious health risks due to HRV. There was no information on the prevalence of HRV in Pakistani primary schools that could be found in the literature review[7,11,13]. Keeping in view of the above situation, the focus of this study is to estimate the risk of HRV diseases through QAMRA tool using reference pathogen (*E-Coli*) to primary school children drinking contaminated water.

MATERIAL AND METHODS

Study Design:

A cross-sectional Study was carried out after Ethical approval from the Institutional Review Board of Muhammad Medical College, Mirpurkhas vide letter No: MMC/3236 on 22-02-2022. All the required ethical standards were followed.

Study Setting and Duration:

The survey was conducted in ten representative districts of Sindh from February 2022 to December 2022. This study was a part of the WASH Project funded by USAID.

Study Population:

The survey was conducted on 425 primary schools in Sindh. Four provinces make up the administrative division of Pakistan. The second-largest province in Pakistan is Sindh, which has a population of roughly 4.8 million and is in the southeast[15]. Sindh has three climatic divisions (units)North, South, and Central Sindh and 29 districts[13,18].

Inclusion and Exclusion Criteria:

All the registered schools with the Education Department Government of Sindh were considered our relevant population[16]. All schools that were unwilling to provide drinking water samples were excluded from the study.

Sample Size:

42,900 primary schools of Sindh was our relevant population that resulted a sample size of 425 primary schools using Open Epi statistical software[17]. The sample size for our study was calculated using: n = [DEFF*Np (1- p)]/ [(d2/Z21-a/2*(N-1)+p*(1-p)] at 95%CI.

Sampling Technique:

A multi stage random sampling was used to identify ten districts from the Sindh province and the schools were selected proportionate to the population size from each district as shown in Fig-1.

Variables of the Study:

The study analyzed the drinking water sample collected from every school. Overall the primary drinking water sources in Sindh province are; groundwater and surface water[11]. However, the water source varies by its regions. In the north, ground and surface water are the main sources of drinking water for Sindh, whereas in the south, surface water is the main source of drinking water for Sindh. The standard technique was used to analyze the water samples.

Data Collection Procedure:

The schools in each district were randomly chosen to provide drinking water samples that would represent the district's overall drinking water quality. Microbial analyses performed to find the

fecal and total coliforms i.e., filtration method and use of EMB and MFC agars (APHA 9222D)[20].

QMRA can portray potential risks from contaminated drinking water; subsequently, necessary water safety management strategies can be applied. However, in developing countries, it is challenging to apply this tool due to limited data on the occurrence of numerous pathogens. One feasible and simple form of QMRA is using data from indicator organisms.

In order to ascertain the HRV ratio to E. coli or total coliform, a thorough literature study was conducted in this regard (Table-1). The table shows that against faecal coliforms and E. coli, respectively, the HAV concentration was 10^4 and the HRV concentration was 10^5, according to the most researchers. This kind of information is useful in settings with limited resources since it takes into account one conversion ratio from indicator pathogen to pathogen with unknown concentration. Thus, considering the available data, in the present study, it is assumed that the ratio for quantifying the pathogen concentration i.e.,1 fecal coliform: 10,000 HAV whereas 1 E-coli: 100,000 HRV. In order to determine the risk of pathogens in drinking water, the viruses were measured by multiplying the ratio with concentration of indicator pathogens[15,21,22].

For the determination of the exposure of HRV in drinking water source, the Exponential Dose-Response Model and β-Poisson model were used. The concentration of HRV was determined from E. coli count number[1].

The *E-coli* Rotavirus is derived through a ratio published in research papers and WHO Health Assessment Report[23].

2	*E-coli*	*Rotavirus*	$1:5_x$ 10^{-6}	$\frac{Pinf}{day} = 1 - \left[1 + \left(\frac{d}{N50}\right)\left(2^{\frac{1}{x}} - 1\right)\right]^{-\alpha}$	24

Table-1: An overview of literature-based HRV risk estimate from signs and pathogens.

E-Coli bacteria were identified in the drinking water samples during this investigation. The average concentration of *E-Coli* bacteria was multiplied by the HRV risk factor to get the concentration of HRV particles in drinking water. Fig-1 Characteristics of study samples. The daily risks of infection for the HRV virus were multiplied by 0.5 to determine clinical disease, and the population's likelihood of illness was multiplied by 0.01% to get the likelihood of mortality[20]. The risk of disease value was multiplied by 1% to determine the likelihood of mortality.

Data Analysis:

Data was entered and analyzed on SPSS version 26. The graphs were developed using Arc GIS version 3. Morbidity and mortality were predicted using the Quantitative Microbial Risk Assessment (QAMRA) model.

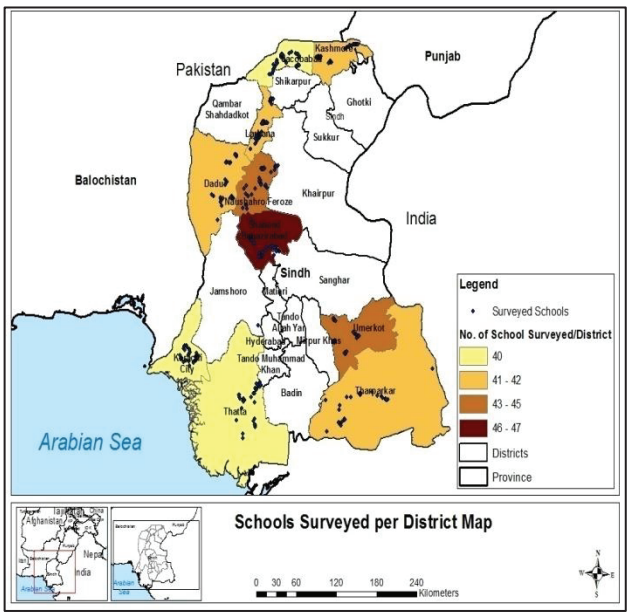

Fig-1: Number of schools surveyed.

RESULTS

The waterborne diseases spread in schools due to poor sanitation and are usually coupled with sewage-contaminated or inadequately treated water. In present investigation, health risks due to HRV associated with infected drinking water in schools are estimated. Although there is a lack of information on the prevalence of HRV infections in Pakistan, a QMRA technique based on indicator pathogens was used to assess the hazards related to HRV exposure. The chance of disease illness, and fatal outcome were calculated in this respect for schools representing 10 different districts of Sindh, with pupils ages 5 to 12, who are consuming untreated contaminated drinking water. Risks were calculated on a daily, yearly, and lifetime basis.

Contamination Of Schools' Drinking Water Sources By Fecal Waste:

The presence of microbes was examined in the collected drinking water samples. The results showed that total and fecal coliform bacteria were substantially polluted in the drinking water of primary schools.

Fig-2:Schools water supply pipes laid parallel with sewerage drains (Right), Excreta discharges into canal & supply pipe (Left)

Fig-2 exhibits the average drinking water condition in primary schools of different districts. The water storage conditions and variations in the water supply led to significant diversity, even within a neighborhood, in the approximately 40–50 schools from each district that were sampled.

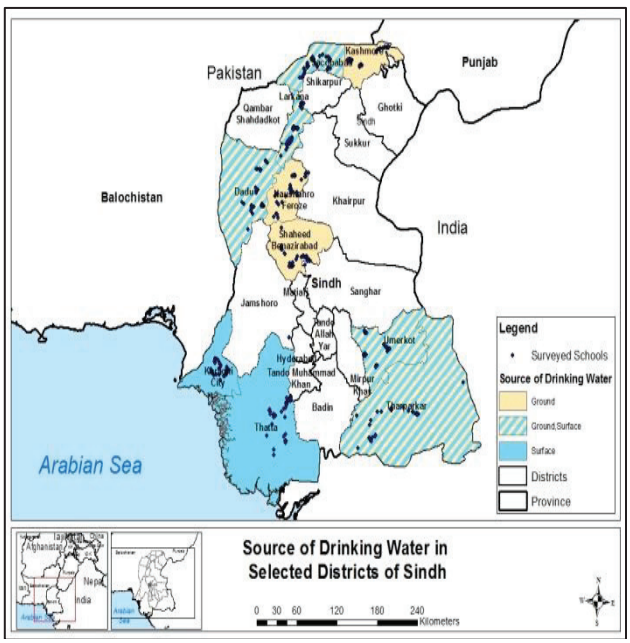

Fig-3: Drinking Water By Type Of Source In Schools

The observed trend for fecal contamination was; Karachi >Umerkot>Dadu>Noushroferoz, whereas the severity of the total coliform contamination was Nousheroferoz>Umerkot> Karachi >Kashmore.

Based on the data, the largest city in Pakistan, Karachi, has the maximum concentrations of *E-coli*, fecal coliforms exposure is equal to Noushroferoz (Fig-2). Larkana had the lowest concentration of fecal coliforms, but it also had the highest concentration of E. coli relative to fecal coliforms. The schools in Kashmore had the lowest E-coli contamination.

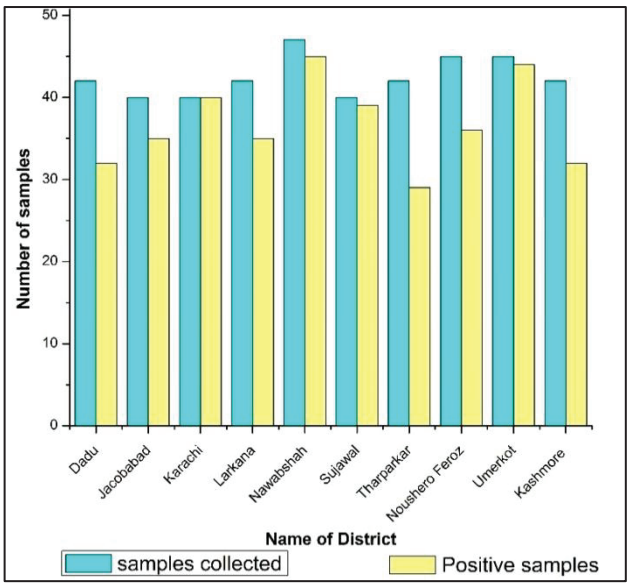

Fig-4: Average Concentration Of Total Coliform Bacteria In Water Samples.

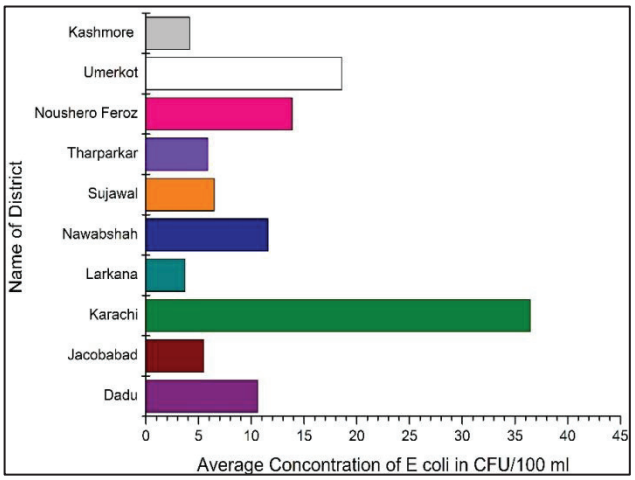

Fig-5: Frequency of samples contaminated with E-coli

HRV Risks to the School Going Children:

The expected daily and annual risks of HRV infections (Table-3), morbidity, and death are shown in Table-3. Children in all 10 districts examined for this study's results are at a high risk of HRV infections. The worst conditions are in the districts of Karachi and Umerkot. The daily risk of HRV infection in Karachi's pupils was estimated to be 11 and 7 in Noushero Feroz, respectively, with an annual risk of 40% and 17.4%, and for the districts Larkana, Jacobabad, Kashmore, Tharparkar, Sujawal, Nawabshah, Noushero Feroz while the daily risk of HRV among districts Umerkot, Nawabshah, Sujawal, Jacobabad, Tharparkar, Dadu, Larkana, Kashmore was found as 6, 3, 2, 2,2, 3, 1 and 1/10,000 pupil having annual risk of 2620, 910, 520, 440, 4700, 1350,300 and 340/10,000 children, respectively. However, Table-3 shows 1250 to 2250 clinical illnesses per 10,000 children. The primary

school students in the districts of Karachi (63%) Noushero Feroz (59%) and Umerkot (45%) had the highest annual probabilities of HRV disease.

Sr. No	District	Average Dose Rotavirus/ day	Prob inf /day	Pinf annual	Prob illness/ year
1	Dadu	53×10^{-5}	3.2×10^{-4}	84×10^{-3}	42×10^{-3}
2	Jacobabad	18.2×10^{-4}	1.6×10^{-4}	44×10^{-3}	22×10^{-3}
3	Karachi	31.01×10^{-4}	10.9×10^{-4}	26×10^{-2}	$13.\times10^{-2}$
4	Larkana	18.5×10^{-5}	1.1×10^{-4}	30×10^{-3}	15×10^{-3}
5	Nawabshah	57.8×10^{-5}	3.5×10^{-4}	91×10^{-3}	46×10^{-3}
6	Sujawal	32.4×10^{-5}	1.9×10^{-4}	52×10^{-3}	26×10^{-3}
7	Tharparkar	29.3×10^{-5}	1.8×10^{-4}	47×10^{-2}	24×10^{-3}
8	Noushero Feroz	69.3×10^{-5}	6.9×10^{-4}	17.4×10^{-2}	87×10^{-3}
9	Umerkot	9.28×10^{-4}	5.6×10^{-4}	$14.2\times1 0^{-2}$	71×10^{-3}
10	Kashmore	20.7×10^{-5}	1.2×10^{-4}	34×10^{-3}	17×10^{-3}

Table-3: Rotavirus probability of illness/year in percent Disease burden due to rotavirus

The burden of diseases Human Rota Virus for rotavirus using QAMRA model shows severity of the diarrhea. The majority of the children are presented with mild diarrhea followed by severe and the probability of death is less than <1%.

Pathogen Rotavirus	Out comes	Severity	Duration	Likelihood of Outcome
Mild Diarrhea	0.1	7	0.02	86%
Severe Diarrhea	0.23	7	0.02	14%
Death from Diarrhea	1	-	56	0.70%
Total				**100%**

Table-4: Estimation of Disease Burden associated with the Rotavirus.

DISCUSSION

India, Nigeria, Pakistan and the Democratic Republic of the Congo contribute to approximately half (49%) of all Rota deaths underage five[10]. Due to poor water sanitation and hygiene facilities, Pakistan contributes to a high risk of oro-fecal pathogen transmission[21]. The Hepatitis A Human entero-viruses (HAV, HEV, HRV) are transmitted principally by the feco-oral route when un-exposed people ingest water contaminated drinking water. In contrast, HRV is considered a major virus causing diarrhea among infants and primary school going children, and it is responsible for around 90% of gastroenteritis in humans globally, with the majority of cases reported in Asia and Africa[6,7].

Children in this study who attended private or public schools (aged 5 to 12) were from middle-class to working-class neighborhoods and drank unfiltered water straight from the source. Thus, the risk of HRV infections is higher. Similarly, a hospital-based study in Pakistan reported that almost 60% of HRV-related diarrheal cases reported are in children below 15 age group[6,26]. People use both groundwater and surface water for drinking in many cities. Sadly, sewage dumping and other industrial influents in the natural water sources have caused these water sources to be contaminated with faecal coliform and other pathogens. Additionally, when there are no microorganisms in the source water, the water becomes contaminated while being stored in overhead tanks. It is known that storage water for drinking purposes is more contaminated with bacteria than water from the source[19].

According to the World Health Organization, children under 15 have a higher prevalence of HRV infections than adults because of their weakened immune systems and poor cleanliness habits[8]. A study in an urban setting of Pakistan revealed that the prevalence of rotavirus was 35% in water samples taken from filtration plants providing drinking water to different settings of the catchment area including schools[7].

The Sindh province also has extremely low levels of enrollment accompanied with high absenteeism, and dropout rates, which have been attributed to inadequate WASH (water supply, sanitation, and hygiene) facilities[15,21]. Primary schools have high levels of interpersonal contact, and overcrowding reflects unhealthy habits and conditions, increasing the risk of rotavirus transmission. Similar studies were mainly performed in hospitals, but a limited one available on a general population[18,19,26].

When there is a lack of information on the presence of pathogens, QMRA estimates can be carried out utilizing the data of indicator organisms, as claimed by Haas et al. But using indicator organisms in QMRA is viewed as a flimsy estimate[20,21]. The existence of *E-coli* in drinking water indicates fecal pollution[11,12]. Additionally, past studies have revealed that the number of faecal coliform bacteria in water source for drinking and recycle waste-water may be the key interpreter of the prevalence of HRV viruses in water used for irrigation and in a recycled drinking water[22,23]. The United States of America Environmental Protection Agency deemed it acceptable to have one waterborne infection per 10,000 customers per year for drinking water[27]. Unfortunately, the current investigation found that primary schools' drinking water quality has frightening conditions.

Additionally, the levels of faecal coliform in school water supplies were higher than permitted. Drinking water should include 0 counts per 100 ml[18,23]. A risk of 37 or 33 per 10,000 school children, or 63.9%, was determined to be the annual risk of HRV.

Although HRV infections are common in poor-income countries, they are the leading cause of severe gastroenteritis at an early age, and its severity decreases with age and with reinfection[28]. This has substantial health, social, and economic consequences for the children, families and the country at large.

Various studies have determined that the drinking water situation of Karachi city is the most critical due to bacterial contaminations[2,9,10,18]. In agreement to this observation, we also found that the children of primary schools of Karachi are at severe risk of HRV associated diarrheal illnesses. The daily highest fecal contamination and load of E-coli was detected in Karachi. Due to the very ancient sewer infrastructure that is intertwined with the water supply system in this city, the amount of fecal contamination in the drinking water is extremely high. After Karachi, 270 per 10,000 school children of Umerkot and 33 per 10,000 school children of Neushero Feroz have critical situation due to HRV related health risks, respectively. According to the results section, the other districts are also experiencing a decline in the quality of their drinking water. Therefore, it comes as no surprise that diarrhea connected to HRV and HAV infections are considered widespread in Pakistan; 90% of children contract HAV before turning 10 years old, primarily as a result of inadequate water quality, sanitation, and hygiene standards[7,18,26]. Additionally, vaccination for HRV is not part of the childhood immunization schedule in Pakistan[15,21].

The estimated annual risk for mortality among children appears to be quite high also, ranging from 2490 to 4500 per 10,000 kids per year. Depending on age, immunological health, and socioeconomic circumstances, mortality rates can range from 0.5% to 1.5%[25].However, it is important to remember that kids who drink two liters of untreated, fecal-contaminated water every day will start to build up their resistance at a young age, growing the projected risk of yearly-deaths from HRV for young kids with weakened immune systems. *E-coli* was initially detected in the water samples (Table-3). Hundred cc of *E-coli* cfu, which is much greater than the WHO or Pakistan[23] drinking water limits, have been found in various districts. Therefore, the WHO or Pakistani requirements were not followed by the water sources in schools[13,23].

Rotavirus risk assessment in educational settings requires more precise data on prevalence rates, virus concentrations in water sources, infectious doses, and recovery skills. To fully capture the risk of HRV infections in educational settings, this study will be extended to incorporate several inputs for the characteristics indicated above. Additionally, by using risk assessment models, other similar viruses, like the hepatitis E virus and adenovirus, could be measured as pathogens in water sources. Since HRVs infections are endemic in Pakistan, all confounders must be considered for a thorough risk analysis. The most important variables are HRV isolation and identification using currently available laboratory techniques; these practical limitations have an effect on the modelling predictions for viral concentration. Although there is no one model for risk assessment, adaptable models should be revised to account for the pathogen, socio-cultural practices, and economic practices of the locals[29]. The study population is vulnerable to serious health risks due to HRV. There was no information on the prevalence of HRV in Pakistani primary schools that could be found in the literature review[7, 11, 13]. In this investigation, the risk of HRV infections in primary school setting is determined by the ratio between the pathogen and indicator organisms thus, this data is not universal, and it only reflects the possible risk of HRV infection in children of primary schools of Sindh, Pakistan. Importantly the model is articulated on overestimates due to uncertainties, yet the calculated risk of HRV infection could be underrated.

CONCLUSION

The primary goal of this study was to estimate for the first time the danger of one major entero-virus by focusing on the magnitude of rotavirus illness and its possible adverse effects on students' academic performance for example enrollment, dropout, absenteeism and illness in Sindh province's primary schools. The resources for water, sanitation, and hygiene (WASH) are interconnected, therefore one resource has an impact on the others either directly or indirectly, according to sustainable development goal six (SDG-6). Therefore, to stop the spread of enteric viruses by assuring the availability of safe drinking water supplies in school settings, there is an urgent need to invest in schools WASH facilities.

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