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Serological Detection of Rotavirus Among Children with Diarrhea in Relation to Different Environmental Conditions

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Abstract: Rotaviruses are the single most important etiologic agents of severe diarrhea of infants and young children worldwide. In the present study, serological detection of rotavirus was done using an enzyme linked immunosorbent assay (ELISA), on 247 stool specimens. These were collected from children with acute diarrhea attending the outpatient clinic of Alexandria University Children's Hospital at El-Shatby, from October 2005 to April 2007. Rotavirus was detected in 33.6% of the collected samples; no specific age group or sex predilection was observed. It was presented with a marked seasonal peak during autumn and winter (58.3% and 40.5%, respectively). Rotavirus was found to be infecting most commonly under-weight children (46.9 %) resulting into fluid loss and severe dehydration (80%). Rotavirus acute gastroenteritis was found to be associated with fever (38.8%), vomiting (39.9%), watery stools, and long duration of diarrheal episodes lasting from one up to six days. The appearance of convulsions among rotavirus-positive cases even in the absence of fever (84.6%) was an important finding. Exclusive formula-fed infants appeared to exhibit the highest disease incidence (50%) while exclusive breast-fed infants had a lower incidence level (35.2%) of the disease. The virus was found to be significantly affecting children living in rural areas of Egypt (43.8%) rather than urban ones (26.1%). Environmental factors that were shown to affect the disease incidence include: the presence of impurities in water (41.6%), broken pipes (58.1%) and water tanks (58.7%) at the residence place. On the other hand, neither the kind of water source nor the presence of a sewage-disposal network was significantly related to the disease. Therefore, the study recommended to screen for rotavirus in children with diarrhea in order to avoid the use of unnecessary medications. In addition, encouragement of breast feeding practices and improvement of environmental conditions are important means of prevention of rotavirus infection.

Key words: *Rotavirus; Diarrhea; Dehydration; Environmental Conditions*

INTRODUCTION:

Rotaviruses have been recognized as the major etiologic agents of acute gastroenteritis in infants and young children worldwide, with Group A rotaviruses being the single most important cause of severe acute diarrhea in young children throughout

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the world.[1] Data presented at the 6th International Rotavirus Symposium in Mexico City, indicated that the annual number of deaths due to rotavirus infection may be as high as 608,000 deaths per year, 82% of which occur in developing countries.[2] The high disease burden motivated major efforts to develop a suitable rotavirus vaccine. However the vaccine efficacy is being challenged by the extensive strain diversity. This made rotavirus a prime target for global estimation of the prevalence and type distribution of the virus strains all over the world.[3,4]

Rotaviruses are classified as a genus in the family *Reoviridae*. The rotavirus genus currently has five species (Rotavirus A to Rotavirus E), with two possible additional species (Rotavirus F and Rotavirus G) based on serological identification of common antigens. Most human infections are caused by group A rotaviruses, although groups B and C rotaviruses have occasionally been associated with human illness.[5]

Rotaviruses are non enveloped, medium-sized viruses ranging from 60 to 80nm in diameter with an icosahedral symmetry. They are composed of 11 double-stranded linear RNA segments surrounded by three concentric spherical protein coats. The inner coat, made of VP2, encloses the genomic RNA and another two minor proteins, VP1 and VP3. The middle layer of the mature viral particle is composed of VP6 polypeptides, which determine group specificity. The outer capsid of rotaviruses is made up of the VP7 glycoprotein and the VP4 hemagglutinin proteins. Serotype specificity is determined by the outer capsid proteins VP4 and VP7, both of which independently induce neutralizing antibodies, thus classifying rotaviruses into G (VP7) and P (VP4) serotypes.[6,7]

The predominant mode of rotavirus transmission is fecal-oral. The infectious particles are shed in high concentrations in the stools of infected children up to one week after infection or for more than 30 days in immunocompromised patients.[8] Spread through respiratory secretions, person-to-person contact, or contaminated environmental surfaces has been also speculated.[9,10] Animal-to-human transmission does not appear to be common, although human rotavirus strains that possess a high degree of genetic homology with animal strains have been identified.[10]

Rotaviruses can survive for weeks in potable and recreational waters and for at least four hours on human hands. The viruses are relatively resistant to commonly used hard-surface disinfectants and hygienic hand-wash agents.[11] In addition, their massive excretion begins with the first day of diarrhea.[12] Although they are found in wastewater and can also be concentrated by shellfish; rotaviruses have not been linked with infectious disease following seafood consumption.[13] In case of improper wastewater management, these large numbers of viruses are usually carried to the surface and ground waters and consequently become a source of infection to those who are drinking, using and in contact with contaminated waters.[14]

Sewage sludge is a complex mixture of solids removed from wastewater in sewage treatment plants. It is the most hazardous by-product in wastewater treatment facilities. The type of treatment determines the concentration of pathogens and the relative risks associated with the produced sludge. Viruses are present in high numbers in raw wastewater and current water treatment practices fail to ensure the complete removal of viral pathogens; consequently, viruses become environmental pollutants.[15]

Solids-associated viruses in wastewater effluents are discharged into aquatic environments and accumulate in the sediments where they persist longer than in the surface water.[16] As a matter of fact, sediments act as a reservoir from which viruses are re-suspended in the water by several natural or artificial phenomena.(17,18)

Accordingly, humans are exposed to enteric viruses through one or more of the various transmission routes such as eating of fresh vegetables grown in contaminated land,
bathing in sewage-polluted recreational waters and drinking contaminated water. (14)

Aim of the work:

The aim of the present work was to study the occurrence of rotavirus infection as a cause of diarrhea among children under 5 years of age in relation to different environmental conditions.

Material and Methods:

The present study was conducted in the period from October 2005 through April 2007. It included 247 stool samples collected from children less than 5 years old, suffering from acute diarrhea, attending Alexandria University Children's Hospital at El-Shatby.

All patients were subjected to thorough history taking from mothers and clinical examination at the time of sample collection. An interview questionnaire was designed to obtain data regarding the name, date, age, sex, type of feeding, duration of diarrhea till the time of sample collection, frequency of motions per day, the presence or absence of fever, vomiting, abdominal colic, flu-like symptoms, convulsions and breast feeding. Also questions regarding the residence, water-source, presence or absence of broken water pipes, water tanks or impurities in drinking water, means of sewage disposal, were also included in the questionnaire. In addition, the body weight and degree of dehydration of each case was clinically assessed.(19)

After taking the parents' consent, stool samples were taken from each child in a clean container. The samples were transferred on the same day to the laboratory where they were stored at -20°C until being tested for the presence of rotavirus using the RIDASCREEN® Rotavirus (C 0901) ELISA kit. Test kit was supplied by R-Biopharm AG (Landwehrstr. 54, D-64293 Darmstadt, Germany). It is an in vitro diagnostic test developed for the qualitative determination of rotaviruses in stool samples using monoclonal antibodies against a capsid protein of gene 6 (VP6).[123] The manufacturer's instructions were closely adhered to in performing the test.

Computerized tabulation was done using SPSS statistical program. The test used was paired sample – T test. Significance was taken at 5% (0.05) level. Values ≤ 0.05 were considered statistically significant.[20]

Results and discussion:

The World Health Organization (WHO) has stated that in considering routine immunization against rotavirus, countries will need sound epidemiological data to assess the burden of disease, to examine trends and seasonality, to evaluate the age patterns of incidence and to determine the serotypes of strains currently in circulation.[21] The present study was an attempt to participate in the research efforts done for estimation of the occurrence of rotavirus diarrhea among children in Egypt. In addition the study examined the incidence of rotavirus diarrhea throughout the four seasons of the year, the clinical features of the disease, and the relation between breast feeding, the place of residence as well as different environmental conditions regarding the presence or absence of the infection.

Rotavirus was identified in 83 (33.6%) of 247 stool specimens collected from children with acute diarrhea who attended the outpatient clinic in Alexandria University Children's Hospital at El-Shatby, over a period of 18 months starting from October 2005 till April 2007. The obtained results showed remarkable agreement with a study done by Radwan *et al.,*[22] who found that 35.6% of 180 stool specimens collected from neonates and infants attending Cairo University Children's Hospital with acute diarrhea during the period from August 1992 till October 1993, were positive for rotavirus. Another study was initiated to determine the prevalence of selected pathogens associated with diarrheas among the children living in El Fayoum Governorate located in south east of Cairo, between August and September 2003. Rotavirus was responsible for 17% of cases with diarrhea among 356 children aged ≤ 6 months.[23] This may be related to the difference in environmental conditions in each study.

Internationally, the same results were reported in a surveillance study done in Venezuela over five years period (1998–2002), where 33% of the stool specimens collected from children admitted to hospital with severe diarrhea were positive for rotavirus.[24] Another surveillance system done during the same period (1998–2002) in Spain by Sànchez-Fauquier *et al*.[25] found that rotavirus was responsible for 31% of acute diarrhea in children less than 5 years old. Moreover, in 2002 Coluchi *et al*.,[26] published that 31.8% of stool specimens obtained from children (mostly under the age of 3 years) with acute diarrhea, collected in Paraguay between January 1999 and March 2000 were positive for rotavirus infection. In another study done during the period of 1980 to 1993 in Melbourne, Australia by Barnes *et al.,*[27] rotavirus was detected in 39.6% of children less than 5 years old who were admitted to the hospital with acute diarrhea, which confirms the huge disease burden all over the world.

Statistical analysis of the obtained results showed that neither different age groups nor gender made a statistical significance in terms of the prevalence of rotavirus among the studied cases as shown in table (1 and 2). It may be due to the small sample size.

Although rotavirus was detected in each season all over the year, there was a strong significant association (P=0.000) between the seasonal trend and the incidence of rotavirus infection as shown in table (3). The highest rate of rotavirus infection (58.3%) was represented in autumn, followed by winter where rotavirus was detected in 40.5% of the diarrheal cases collected during the season. Rotavirus infection was detected in 33.3% of the cases collected during the summer season, and the lowest incidence of rotavirus infection was found in spring (10.2% of the cases).

A marked seasonal peak during the cold seasons of the year (autumn and winter), was also observed in England,[9] Finland,[28] Spain[25] and Tunisia.[29] This was also similar to a previous cohort study conducted in Bilbeis (Egypt), where the rate of rotavirus isolation predominated in the colder months (November – April).[30] However, the seasonal peak of rotavirus infection in Egypt tends to shift over consecutive years.[22] In a previous population-based cohort study of children less than 3 years of age residing in Abu Homos in 1995 till 1996, rotavirus infection predominated during the warmer months (July – November), with a peak incidence in August.[31] Moreover, rotavirus predominated from August to December during the period of 1992–1993 as published by Radwan *et al*.[22]

These variations in the timing of the peak rotavirus activity in Egypt have been also reported in other settings all over the world. It recalls the patterns observed in less developed countries with a tropical climate, in which seasonality of rotavirus infection is unclear or nonexistent.[32,33]

In table (4), there was a significant association between rotavirus infection and body weight of affected children (P=0.027).

Rotavirus was found to be infecting most commonly under-weight children (46.9%), while it was found in 30.3% of normal-weight children.

As regards the duration of diarrhea, table (5) represents a statistically significant association (P=0.05) between rotavirus positive and negative cases in relation to the duration of diarrhea. The duration of diarrhea ranged from one day to more than 7 days at the time of sample collection. Rotavirus was detected in 33.3% of the cases having diarrhea for one day, in 27.3% of the cases having diarrhea for 2 days and in 32.6% of the cases having diarrhea for 3 days. The highest percent of positive cases were found to suffer from diarrhea which lasted between 4 days (51.4%) and 5 days (50%) at the time of sample collection. Twenty five percent had diarrhea for 6 days and none of the cases were found to have diarrhea for 7 days or more.

The clinical features associated with rotavirus infection are shown in table (6). There was no significant association between rotavirus infection and the presence or absence of abdominal colic (P=0.799), fever (P=0.059), bloody stools (FETp=0.667) or flu-like symptoms (P=0.417). On the other hand, rotavirus infection was significantly associated with vomiting (P=0.002), severe dehydration (MCP=0.000) and convulsions even in the absence of fever (P=0.000).

A one year study of the etiology of acute diarrhea complicated by severe dehydration, active bleeding, shock and cardiovascular collapse, pneumonia, acute renal failure, or seizures was performed in Cairo, Egypt in 1986 on 145 infants. A variety of enteropathogens were identified with approximately equal frequency in the fatal and nonfatal complicated cases as well as in 135 controls with severe uncomplicated diarrhea. Rotavirus was among the agents most frequently detected in infants with severe diarrhea in this population representing 33%, followed by heat-stable enterotoxin-producing *E. coli* in 20% of cases.[34]

It is worth notice that in the present study, eleven of the rotavirus-infected children suffered from convulsions that were not associated with fever. A remark consistent with several some case reports that found evidence of rotavirus RNA by PCR technique in the cerebrospinal fluid of rotavirus-infected children who had seizures[35] and in liver and kidney sections of immunocompromised children.[36] Another study used RT-PCR, immunohistochemistry, and in situ hybridization to detect rotavirus in a variety of internal organs of two children who died with severe rotavirus-associated diarrhea and who also had neurological disease.[37] One elegant study used an EIA test designed to detect rotavirus antigen in stool samples but applied it to serum and found that 22 out of 33 immunocompetent children with confirmed rotavirus gastroenteritis had rotavirus antigenemia, providing evidence that rotavirus may commonly escape the gastrointestinal tract. The clinical significance of these findings remains unclear but is under active investigation.[38]

In table (7), the highest percent of rotavirus positive cases was found among infants who were exclusively formula-fed, while those who were exclusively breast-fed showed a lower incidence level (50% Vs 35.2%). Infants who received both breast and formula feeding had the least percent of positive cases (22.6%) and those who received neither (children more than 2 years old) showed 25.9% of rotavirus positivity. However, this difference did not reach a statistically significant level. (P=0.062)

Our results were coincident with previous studies done in Egypt[39] and Bangladesh[40]; breast feeding was associated with a lower incidence of rotavirus diarrheal episodes, which add to the multitude of benefits that have been associated with breast feeding. Moreover, it was reported that early initiation of breastfeeding was associated with a marked reduction of the rate of diarrhea throughout the first 6 months of life, possibly because of the beneficial effects of human colostrum.(39)

Table (8) shows that rotavirus infection was found to be significantly affecting children living in rural areas (43.8%, P=0.003) rather than those who are living in urban areas (26.1%). Poor sanitation mainly means absence of integrated wastewater and solid waste management systems and absence of sanitary drinking water supply, which are common in Egyptian rural areas. In around 95% of Egyptian villages, wastewaters and solid wastes find their way to small canals and drains which are used in irrigation of fresh vegetables and other crops.(41) Eating of fresh vegetables which are grown in contaminated land irrigated by contaminated water without proper washing or even washed by contaminated water could be a possible source of infection with rotavirus. Also, farmers who are being in contact with contaminated land and water during day time, using the same source of water to get washed at the end of the day, then getting in contact with their children in the evening is one point to be kept in mind when considering possible sources of rotavirus infection.

Table (9) demonstrates a statistically significant association between rotavirus infection and the existence of broken drinking water supply pipes (58.1%, P=0.000), presence of water storage tanks (58.7%, P=0.000) and presence of impurities in water at the residence place (41.6%, P=0.001). On the other hand, the source of water (P=0.451) and the means of sewage disposal (P=0.295) were not found to be statistically significant as regards the presence or absence of rotavirus.

Contaminated drinking water is one of the most important sources of infection with rotavirus. The main causes of drinking water contamination are contaminated raw water sources (surface or ground water) accompanied with inefficient water purification and disinfection processes. In addition, interrupted drinking water pumping accompanied with the existence of broken pipes in drinking water distribution network leads to negative pressures and consequently entry of contaminants and impurities into the drinking water networks. The presence of impurities in drinking water may act as a good media for the growth of different microorganisms especially in the absence of residual disinfectant (chlorine gas) which normally occurs at the end of drinking water networks (usually in rural areas). Presence of impurities in water, consumption of water from water-tanks and the existence of broken pipes at the residence place, are findings that fully agree with the results documented by Zaki *et al*.,[30] in 1986.

Another study done in Pakistan in 1992 reported that most of the positive cases were collected from those living in remote rural areas of the country, where increased family size and over crowding were common factors.[42] These are significant risk factors for the mode of transmission of rotavirus due to environmental contamination and contact with the hands of infected persons.[43]

The role of water in the transmission of viruses is well established. However, the epidemiological evidence of water-borne transmission of human viruses is limited to only few including rotavirus.[44] The work done in Pakistan was designed to examine whether or not rotaviruses were present in the influents and to evaluate the virological quality of water as delivered to the consumers in different localities of Karachi. The results showed the presence of rotavirus in 60% influent samples. It was also found that some of the tap-water samples collected had shown the presence of fecal coliforms, suggesting that the water source was also polluted due to either the intermittent water supply or to a leakage in the distribution pipelines or in the nearby sewage lines. Because of these factors, drinking water must be regarded as having a very significant potential as a vehicle for the transmission of rotaviruses. Therefore, the most reasonable approach for controlling the transmission of viruses through water is to recommend additional parameters to be added to the safety criteria of drinking water.[42]

Nevertheless, we could not adequately account for several factors that might have affected our findings. First, the study was conducted in a selective population that may not have been representative of the entire country. Second, in our pilot study, the vast majority of the patients refused to give any data concerning their socio-economic status (such as monthly income, number of rooms and number of people living in the house…etc.) Finally, the results of the present work underline the importance of continued detailed epidemiological, environmental and virological studies to identify rotavirus serotypes that cause severe gastroenteritis, including characterization of less common and unusual strains. Knowledge of rotavirus prevalence and strains circulating in our community will aid in assessing the suitability of candidate vaccines, in order to protect against all currently circulating rotavirus strains.

Conclusion and Recommendations:

The present study confirms the huge burden of rotavirus as a major cause of acute diarrhoea in Egyptian infants and young children. The disease was predominant in autumn and winter and was characterized by vomiting, watery diarrhea and severe dehydration. Breast-fed infants were more protected against the disease than those who were exclusively formula-fed. Residence in rural areas of the country and the quality of drinking water had an effect on the occurrence of the disease. Accordingly, we recommend rotavirus screening in children with diarrhea in order to avoid the use of unnecessary medications. In addition, encouragement of breast feeding practices, as well as, improvement of environmental conditions is recommended as means of prevention of rotavirus infection. Finally, the presence of convulsions among rotavirus-positive cases was an important finding and requires further investigation.

Table (1): Distribution of rotavirus infection among children with diarrhea according to the age, Alexandria, 2005-2007.

Age in months	Rotavirus infection				Total	
	Positive (83 cases)		Negative (164 cases)			
	No.	%	No.	%	No.	%
< 6 months	26	31.7	56	68.3	82	100.0
6-	26	33.8	51	66.2	77	100.0
12-	22	47.8	24	52.2	46	100.0
18-	4	50.0	4	50.0	8	100.0
24 to 60 months	5	14.7	29	85.3	34	100.0
Mean ± SD	10.58 ± 8.71		11.99 ± 12.19		11.51 ± 11.14	
Median	8.00		7.50		8.00	
Test of significance	t = 1.05			P = 0.297		

Table (2): Distribution of rotavirus infection among children with diarrhea in relation to gender, Alexandria, 2005-2007.

Gender	Rotavirus infection				Total	
	Positive (83 cases)		Negative (164 cases)			
	No.	%	No.	%	No.	%
Male	50	30.5	114	69.5	164	100.0
Female	33	39.8	50	60.2	83	100.0
	X^2 = 2.12			P = 0.145		

Table (3): Seasonal distribution of rotavirus infection among children with diarrhea, Alexandria, 2005-2007

Season	Rotavirus infection				Total	
	Positive (83 cases)		Negative (164 cases)			
	No.	%	No.	%	No.	%
Autumn	21	58.3	15	41.7	36	100.0
Winter	30	40.5	44	59.5	74	100.0
Spring	6	10.2	53	89.8	59	100.0
Summer	26	33.3	52	66.7	78	100.0
	$X^2 = 25.99$			P = 0.000*		

* Significant

Table (4): Distribution of rotavirus infection among children with diarrhea in relation to body weight, Alexandria, 2005-2007

Weight	Rotavirus infection				Total	
	Positive (83 cases)		Negative (164 cases)			
	No.	%	No.	%	No.	%
Under-weight	23	46.9	26	53.1	49	100.0
Normal weight	60	30.3	138	69.7	198	100.0
	$X^2 = 4.87$			P = 0.027*		

* Significant

Table (5): Distribution of rotavirus infection among children with diarrhea in relation to duration of diarrhea at the time of sample collection, Alexandria, 2005-2007.

Duration of diarrhea in days	Rotavirus infection				Total	
	Positive (83 cases)		Negative (164 cases)			
	No.	%	No.	%	No.	%
One day	10	33.3	20	66.7	30	100.0
Two days	15	27.3	40	72.7	55	100.0
Three days	15	32.6	31	67.4	46	100.0
Four days	18	51.4	17	48.6	35	100.0
Five days	12	50.0	12	50.0	24	100.0
Six days	13	25.0	39	75.0	52	100.0
Seven days or more	0	0.0	5	100.0	5	100.0
Total	83		164		164	
Mean ± SD	3.70 ± 1.93		4.37 ± 3.44		4.14 ± 3.03	
Median	4.00		3.00		3.00	
Test of significance	t = 1.95			P = 0.05*		

* Significant

Table (6): The relation between rotavirus infection among children with diarrhea and some clinical presentations, Alexandria, 2005-2007.

Clinical Presentation	Rotavirus infection				Total	
	Positive (83 cases)		Negative (164 cases)			
Abdominal colic	No.	%	No.	%	No.	%
Present	58	34.1	112	65.9	170	100.0
Absent	25	32.5	52	67.5	77	100.0
	X^2 = 0.06			P = 0.799		
Vomiting	No.	%	No.	%	No.	%
Present	67	39.9	101	60.1	168	100.0
Absent	16	20.3	63	79.7	79	100.0
	X^2 = 9.29			P = 0.002*		
Fever	No.	%	No.	%	No.	%
Present	52	38.8	82	61.2	134	100.0
Absent	31	27.4	82	72.6	113	100.0
	X^2 = 3.55			P = 0.059		
Bloody stool	No.	%	No.	%	No.	%
Present	1	16.7	5	83.3	6	100.0
Absent	82	34.0	159	66.0	241	100.0
	FETp = 0.667					
Dehydration degree	No.	%	No.	%	No.	%
No signs	39	24.8	118	75.2	157	100.0
Some dehydration	40	47.1	45	52.9	85	100.0
Severe dehydration	4	80.0	1	20.0	5	100.0
	MCP = 0.000*					
Flu-like symptoms	No.	%	No.	%	No.	%
Present	40	31.3	88	68.8	128	100.0
Absent	43	36.1	76	63.9	119	100.0
	X^2 = 0.66			P = 0.417		
Convulsion	No.	%	No.	%	No.	%
Febrile Convulsion	39	24.8	118	75.2	157	100.0
Afebrile Convulsion	40	47.1	45	52.9	85	100.0
No Convulsion	4	80.0	1	20.0	5	100.0
	X^2 = 46.73			P = 0.000*		

* Significant

Table (7): The relation between breast feeding and rotavirus infection among children with diarrhea, Alexandria, 2005-2007.

Type of feeding	Rotavirus infection				Total	
	Positive (83 cases)		Negative (164 cases)			
	No.	%	No.	%	No.	%
Exclusive breast-fed	45	35.2	83	64.8	128	100.0
Exclusive formula-fed	17	50.0	17	50.0	34	100.0
Both breast and formula-fed	7	22.6	24	77.4	31	100.0
Neither	14	25.9	40	74.1	54	100.0
	X^2 = 7.35			P = 0.062		

* Significant

Table (8): Distribution of rotavirus infection among children with diarrhea among rural and urban patients, Alexandria, 2005-2007.

Residence	Rotavirus infection				Total	
	Positive (83 cases)		Negative (164 cases)			
	No.	%	No.	%	No.	%
Rural	46	43.8	59	56.2	105	100.0
Urban	37	26.1	105	73.9	142	100.0
	X^2 = 7.35			P = 0.062		

* Significant

Table (9): Distribution of rotavirus infection among children with diarrhea in relation to some environmental conditions, Alexandria, 2005-2007.

Environmental conditions	Rotavirus infection				Total	
	Positive (83 cases)		Negative (164 cases)			
Water source	No.	%	No.	%	No.	%
Tap water	79	34.2	152	65.8	231	100.0
Other	4	25.0	12	75.0	16	100.0
	X^2 = 0.57			P = 0.451		
Broken pipes	No.	%	No.	%	No.	%
Present	36	58.1	26	41.9	62	100.0
Absent	47	25.4	138	74.6	185	100.0
	X^2 = 22.20			P = 0.000*		
Water tank	No.	%	No.	%	No.	%
Present	27	58.7	19	41.3	46	100.0
Absent	56	27.9	145	72.1	201	100.0
	X^2 = 15.95			P = 0.000*		
Sewage network	No.	%	No.	%	No.	%
Present	62	32.0	132	68.0	194	100.0
Absent	21	39.6	32	60.4	53	100.0
	X^2 = 1.10			P = 0.295		
Water impurities	No.	%	No.	%	No.	%
Present	64	41.6	90	58.4	154	100.0
Absent	19	20.4	74	79.6	93	100.0
	X^2 = 11.60			P = 0.001*		

* Significant

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