Detection of rotavirus in sewage and drinking water by Latex agglutination test

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Abstract- Rotavirus is mainly associated with gastroenteritis in adults and major cause of diarrhea in child's under 5 years of age. The present study was designed to standardize the latex agglutination test with respect to agar gel precipitation test and then detection of rotavirus in sewage and drinking water by latex agglutination test. Latex agglutination test is an easy, rapid, highly sensitive and specific test for diagnosis as compared to other methods. However, for the current study n= 240 samples were collected by convenient sampling technique from different sources including half (n= 120) from sewage sources and half (n= 120) from drinking water sources. The collected samples were transferred under optimum conditions 4^{o}C temperature in sterile falcon tubes to laboratory of Institute of Microbiology, Faculty of Veterinary Science, University of Agriculture, Faisalabad. Then samples were processed for rotavirus detection by latex agglutination test. The results were analyzed by ANOVA technique using SPSS version 20.0 of computer program. The present study showed 60% (72/120) and 26.6% (32/120) rotavirus in sewage and drinking water respectively. The present study concluded that rotavirus percentage was detected higher in sewage water then drinking water and needs precautions to avoid contamination of sewage water containing higher amount of rotavirus.

Index Terms- Drinking water, Gastroenteritis, Latex agglutination test, Rotavirus, Sewage water

I. INTRODUCTION

Globally, 1.1 billion people cannot access clean drinking water, and 2.6 billion people do not have adequate sanitation facilities, because of this situation, from 4,000 to 6,000 child's are dying daily. Although, viruses are the main cause of waterborne diseases, the health effects of viral infections transmitted through water are underestimated [1]. Human enteric viruses, which primarily infect and replicate in the gastrointestinal tract, have been associated with waterborne transmission. Among the viruses, rotavirus is leading cause of gastrointestinal problems in child's and adults [2] which is quickly transmissible virus further categorized into group A causing diarrhea while group B and C are responsible for adult gastroenteritis. Rotavirus belongs to the family Reoviridae and is an encapsulated virus with a double-stranded 11-segment RNA genome [3]. Rotavirus (RV) is the dominant etiology of gastrointestinal problems of infants all over the world [4] encompassing annual 25 million clinical cases, 2 million hospital, and 0.6 million causalities in infants. The incidence of RV is one third of all hospitalizations for diarrhea, estimated that 4-6 lac deaths per year in children. Major of deaths occurs in developed countries such as Asia and Saharan Africa that is thought to because of delay in hospitalization [5]. Transmission of RV has been testified through consumption of sewage, sea water, and contaminated drinking water that lead to gastric problem in humans. The prevalence of rotavirus varies as 17% in child's < 5 years of age, 22% in hospitalized patients in Gambia [6], 28.15% in India among the hospitalized children's, [7] and 24% in Ethiopia in child's <5 years of age before vaccination [8]. Prevalence of rotavirus in water supplies was detected up to 35% in Karachi, Pakistan pose a serious risk to public health [9]. There are many tests to diagnose the rotavirus. In the past, the diagnostic lab used an electron microscope (EM) to detect a virus.

However, this approach is not favorable due to the cost of the microscope and its maintenance and the necessary technical expertise. In addition, EM lacks sensitivity and requires about 10^6 virions per ml to detect the virus [10]. Immunoassays (for example, ELISA) have replaced EM as a diagnostic test for the selection of viral antigens because of their ease of use and speed of results. However, due to antigen drift, immunoassays are less specific and more expensive [11]. More recently, molecular methods such as real-time RT-PCR and RT-PCR have supplanted other diagnostic assays with higher analytical sensitivity and specificity. However, these types of tests are conducted in commercial or diagnostic laboratories, require expensive equipment and advanced technical capabilities, which leads to an increase in the cost of manufacturers, leads to Less use to support field diagnosis [12]. Latex agglutination test is an assay used clinically in the identification and typing of many important microorganisms [13]. Latex agglutination test is a quick, simple, easily can do in laboratory, with high sensitivity (85.9%) and specificity (97.7%) [14]. Therefore, the present study was designed to detect the rotavirus in sewage and drinking water through latex agglutination test.

II. MATERIALS AND METHODS

2.1. Sample Collection

The water samples for the present study were collected from sewage and suspected contaminated water sources. The number of samples for present study comprised of n=240 (n= 120 sewage water, n= 120 contaminated drinking water) as determined by convenient sampling technique. Collected samples were shipped at 4^o C in sterilized falcon tubes to laboratory of Institute of Microbiology, University of Agriculture, Faisalabad for detection of rotavirus. Collected water samples were two fold diluted in tris buffer (0.02M). These samples were tested for rotavirus by latex agglutination test.

2.2. Standardization of LAT with respect to AGPT

2.2.1. Immunogenicity protocol in rabbits

Six rabbits of age (24 weeks) and weight (1500grams) were purchased from Jhang Bazar Faisalabad, Pakistan were randomly divided into three groups (G1, G2, and C) each having two rabbits. Rota virus vaccine (Rotarix gsk, British) was given orally to G1 at dose rate of 0.2mL, 0.4mL, 0.8mL, 1.6mL with one day interval while 0.2mL, 0.4mL, 0.6mL, and 0.8mL was allocated with same protocol to G2. Control group (C) was received the sterile Phosphate buffer saline (PBS). Blood was collected in sterile centrifuge tubes at day 14 and 30^{th} of last dose of vaccine allocated to rabbits. The serum was separated by centrifugation at 1500 rpm for 10 minutes and stored at -4°C in centrifuge tubes [15].

2.2.2. Antibody detection

Three dilutions of serum were prepared in phosphate buffered saline, separately, with concentration of 17ug/mL, 8.5ug/mL, and 4.2 ug/mL. Four sets of sterile test tubes for each group were labelled with three concentrations made, and the one kept as control. The prepared dilutions were poured into each plate. Agar gel precipitation test was used to check the titer of antibodies as proposed by [16]. Positive samples show a clear line of precipitation around the antigen and negative samples showed no line or incomplete lines. Then standardization of latex agglutination test was also done by taking AGPT as gold standard.

2.3. Latex agglutination test for Rota virus detection

2.3.1. LAT solutions and antibodies coating with latex particles

Latex agglutination test involve the preparation of several types of solutions such as glycine buffer, 0.5M EDTA solution, 1mM EDTA solution, 1% PBS solution, 0.04M NaCl solution 0.02M tris buffer, 1% BSA solution, latex suspension in glycine buffer and antibodies were coated on latex particles by incubating the serum antibodies of different concentrations (17ug/ml, 8.5ug/ml, and 4.2ug/ml) and latex suspension in glycine buffer at 37^o C for 2 hours and then keep it at 4^o C for overnight. Then centrifuge it at 15000 rpm for 20 minutes, discard the supernatant and re-suspend the pallet in 1% BSA in glycine buffer and

incubate it under same conditions. Then wash the pallet 3 time with glycine buffer containing 3% NaCl, 5% sucrose, 5% choline chloride, and 0.02% NaN3 and finally suspend it at 1% concentration for further use.

2.3.2. Latex Agglutination test protocol

A 20µL of 2 fold diluted samples were mixed with equal volume of glycine buffer (20µL) having 1% PBS and 20µL of 1% antibody coated latex particles on clean glass slide. Slide was incubated in moist chamber for 45 minutes. Presence of agglutination indicated positive sample [15]. Comparisons of LAT results were done with that of standardized AGPT.

2.4. Statistical analysis

The data obtained from three groups was subjected to statistical analysis using ANOVA technique, and means were compared by least significant difference test using SPSS version 20. However, the prevalence of rotavirus was determined by formula described by [17]. The level of significance was assessed at 5% probability ($p < 0.05$).

III. RESULTS AND FINDINGS

3.1. Immunogenicity evaluation by LAT

The geometric mean titer of serum antibodies from vaccinated rabbits was detected by quantitative latex agglutination test (table 1, fig 1). Results showed GMT to be significantly higher (p <0.05) in G1 compared to those in groups G2 and C both at 15^{th} and 30^{th} day post last vaccination day.

However, the antibody titer was detected low at 30days of post last inoculation compared to at 15days post last inoculation. However, a non- significant difference ($p > 0.05$) was observed in relation with antibody titer among the immunogenic groups while a significant difference ($p < 0.05$) was observed among the immunogenic groups and control group.

Table 1. Geometric mean titer values of antibody titer in different groups of rabbits

Days serum collected	Geometric mean titer values (GMT)			P- value
	G1	G2	C	
15	7.4 ± 0.14^{a}	6.6 ± 0.21^{b}	6.2 ± 0.14^{c}	0.01
30	7.0 ± 0.21^{a}	6.4 ± 0.14^{b}	5.6 ± 0.35^{c}	0.02

Different superscripts within row indicate significant results ($p< 0.05$)

G1= Group 1, G2= Group 2, C= Control

3.2. Immunogenicity evaluation by AGPT

Agar gel precipitation test was used to detect the presence and absence of antibodies in serum samples obtained from Rotavirus vaccinated rabbits. AGPT showed positive results for all rabbits vaccinated against Rotavirus while showed negative results for control groups (unvaccinated).

3.3. Evaluation of Rotavirus from contaminated drinking water by qualitative LAT

The present study showed 26.6% (32/120) overall positive samples and 73.4% (88/120) over negative samples regardless of single positive, double positive, and three positive. Among the overall 26.6 % positive samples the ratio of single positive samples was highest 62.5% (20/32) followed by 25% (8/32) and 12.5% (4/32) as double positive and three positive, respectively (fig. 1).

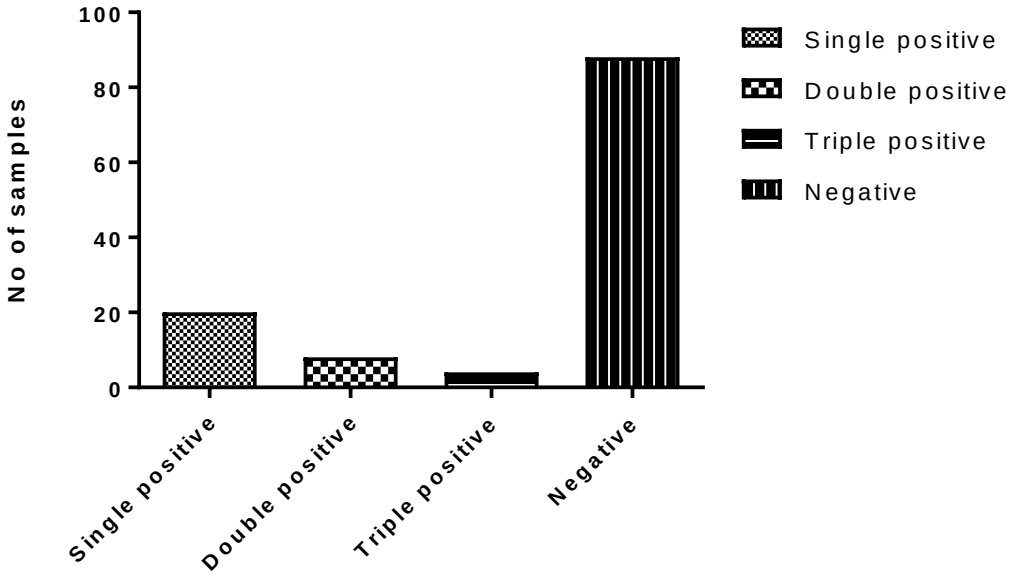

Figure 1. Number of samples positive and negative for Rotavirus in contaminated drinking water

3.4. Evaluation of Rotavirus from sewage water by qualitative LAT

The present study showed 60% (72/120) overall positive samples and 40% (48/120) over negative samples. Among the overall 60 % positive samples the ratio of single positive samples was highest 44.4% (32/72) followed by 27.8% (20/72) and 27.8% (20/72) as double positive and three positive, respectively (fig. 2).

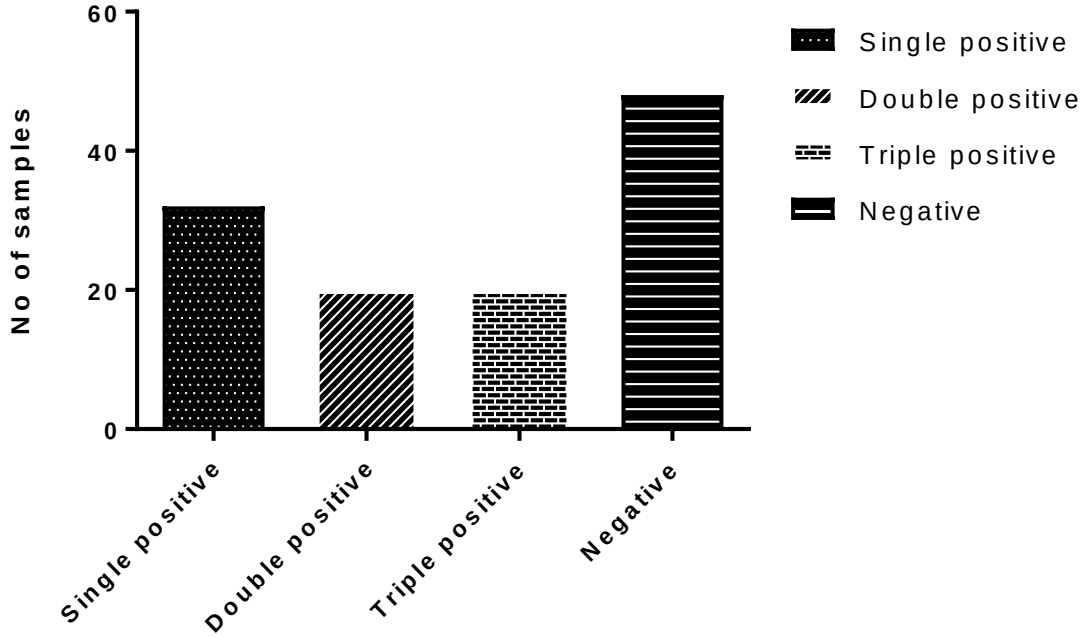

Figure 2. Number of samples positive and negative for Rotavirus in sewage water

3.5. Comparison of Rotavirus prevalence in contaminated drinking and sewage water

The current study showed overall highest rate of Rotavirus in sewage water 60% (72/120) as compared to contaminated drinking water 26.6% (32/120). However, the differences also exist in their positivity levels among contaminated drinking water and sewage water. (Fig. 3).

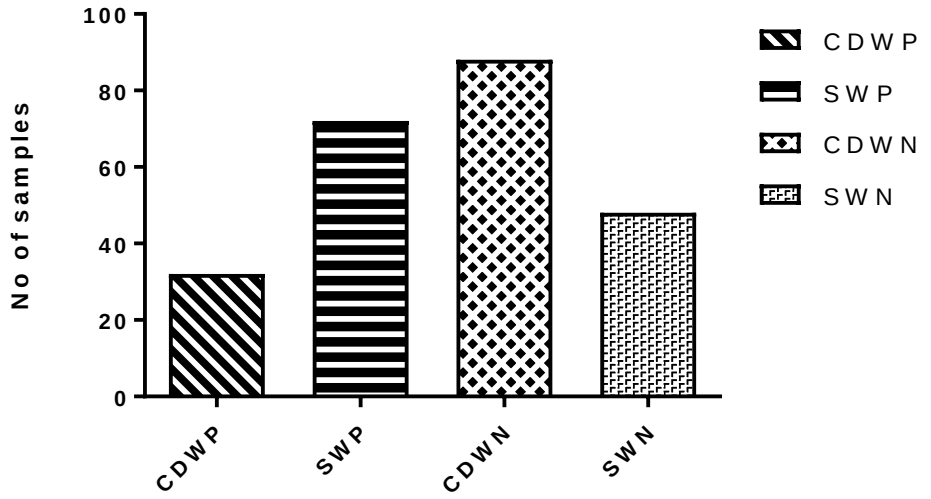

Figure 3. Comparison of Rotavirus in contaminated drinking sewage water

CDWP= Contaminated drinking water positive, SWP= Sewage water positive, CDWN= Contaminated drinking water negative, SWN= Sewage water negative

IV. DISCUSSION

Replication of an enteric rotavirus can result in more than 10^{10} infectious particles per gram of feces excreted by symptomatic and asymptomatic individuals. Therefore, the content of these viruses in wastewater reflects the status of infection of the population. Thus, an environmental sample of raw wastewater is a suitable method for studying enteroviruses circulating in a particular population [18]. In present study high prevalence rate of rotavirus in sewage water was detected that is similar with findings of [19] while prevalence of rotavirus in contaminated drinking water is in agreement with findings of [9]. The present study shows that high prevalence of rotavirus was present in sewage water because of fecal material present in sewage water that contain high amount of rotavirus while drinking water can be contaminated with enteric viruses by a variety of sources, including raw and treated sewage, wastewater discharges, animal manure and unprotected connections [20]. However, the present study findings of rotavirus in sewage water are not in agreement with findings of [18] that may due to use of different techniques for rotavirus detection having different sensitivity and specificity. The present study findings of rotavirus in sewage water is highly conflicting with findings of [21]. This discrepancy among the results may be due to use of different techniques for rotavirus detection.

V. CONCLUSION

Latex agglutination test is simple, cost effective and a rapid technique to identify the quality of water with in short period of time. This test can be done easily in lab with no such highly qualified staff is required. However, rotavirus prevalence was detected high in sewage water as compared to drinking water that indicative of contamination of drinking water with sewage water and need much attention to avoid a list of diseases.

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