Rotavirus and adenovirus in human and animals in Southwest of Saudi Arabia

Abuelyazeed A. Elsheik[1], Walid A. Azab[2], Abdulrahman M Al-Qurashi[3] and Shimaa M.G. Mansour[4]

[1]Department of Applied Medical Sciences, Community College, Najran University, Najran, Saudi Arabia. Department of Virology, Faculty of Veterinary Medicine, Zagazig University, Egypt.
[2]Institut für Virologie, Freie Universität Berlin, Philippstrasse 13, Haus 18, D-10115 Berlin, Germany. Department of Virology, Faculty of Veterinary Medicine, Zagazig University, Egypt.
[3]Department of Biology, Faculty of Science, Najran University, Najran, Saudi Arabia
[4]Department of Virology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt
eaa000@yahoo.com

Abstract: Enteric viruses are important agents threaten both human and animal health. These viruses are usually transmitted via the fecal-oral route and are shed in extremely high numbers in the feces of infected individuals. This study was carried out to determine the prevalence of rotavirus and adenovirus infections among humans and animals in Najran region (a province located at the southwest of Saudi Arabia) and to identify the source of infection. A total of 92 and 88 stool samples were collected from children and lambs suffering from diarrhea, respectively. All stool samples were tested with two antigen detection techniques; (ELISA) and RIDA QUICK Rotavirus/Adenovirus Combi for detection of rotavirus and adenovirus. The positive results were further confirmed by PCR. To identify the source of infection, five potable water samples were tested for both viruses by PCR technique. In children, the results showed that 8 samples were positive for rotavirus (8.69%), while 3 samples were positive for adenovirus (3.26%). In lambs, there were 4 positive samples for rotavirus (4.54%) while the adenovirus could not be detected in any of the samples. The viruses could not be detected in any water sample. This is the first study that shows the presence of enteric viruses in humans and animals in Najran and further investigations are needed to identify the source of infection.

[Abuelyazeed A. Elsheik, Walid A. Azab, Abdulrahman M Al-Qurashi and Shimaa M.G. Mansour **Rotavirus and adenovirus in human and animals in Southwest of Saudi Arabia.** Journal of American Science 2012; 8(2):489-493]. (ISSN: 1545-1003). http://www.americanscience.org. 67

Key words: Rotavirus, Adenovirus, ELISA, Saudi Arabia

1. Introduction

Enteric viruses refer to an important, but diverse, group of viruses found in the intestinal tract of humans and animals ***(Theil, 1990; Wilhelmi et al., 2003; Fong and Lipp, 2005)***. These viruses are the most common cause of childhood gastroenteritis over the world ***(Wilhelmi et al., 2003; Weitzel et al., 2007)***.These viruses can be spread in the environment through groundwater, seawater, rivers, aerosols emitted from sewage treatment plants, insufficiently treated water, drinking water, and private wells that receive treated or untreated wastewater either directly or indirectly ***(Bosch, 1998; Lee and Kim, 2002; Griffin et al., 2003)***. Enteric viruses have been isolated from contaminated drinking water sources, recreational waters, urban rivers, and shellfish harvested from contaminated waters. In the environment, enteric viruses can survive under a wide pH range (pH 3 to 10) and for extended periods at low temperatures. Among the enteric viruses, rotaviruses and enteric adenoviruses 40 and 41 are considered the most important ones ***(Jiang et al., 2001; Jiang, 2006)***.

Rotaviruses are members of the *Reoviridae* family and are characterized by their non-enveloped icosahedral structure with 70-nm diameter. Rotaviruses are classified according to the antigenic properties of the group reactivity determinant VP6 capsid protein into seven groups, A to G, and two subgroups I and II ***(Estes and Kapikian, 2007)***. Group A rotaviruses are the major cause of diarrhea in young children and animals worldwide (***Puig et al., 1994; Weitzel et al., 2007)***. Several European studies pointed to rotavirus A as the agent responsible for 20-60% of cases of gastroenteritis (***Wilhelmi et al., 2003)***. In Saudi Arabia, rotavirus is an important cause of severe diarrhea among children. The prevalence of rotavirus infection ranged from 10 to 46% with an average of 30%. Most of the cases were among children less than 2 years of age, and particularly in the first year of life ***(Al-Bwardy et al., 1988; Al-Freihi et al., 1993; Ghazi et al., 2005; Kheyami et al., 2006)***.

Adenoviruses belong to the double-stranded DNA *Adenoviridae* family. Adenoviruses types 40 and 41 are recognized as important etiologic agents of gastroenteritis in children; however, they are second after rotaviruses as a cause of diarrhea ***(Allard et al., 1992; Horwitz, 1996; Crabtree et al., 1997; Jiang, 2006)***. The enteric adenoviruses, types 40 and 41,

cause mortalities as much as 50% in immunocompromised individuals ***(Hunter, 1997)***. Transmission of these viruses includes the fecal-oral route and inhalation of aerosols. The viruses are shed for extended periods in feces, urine, and respiratory secretions of infected persons ***(Ishibashi and Yasue, 1984; Cruz et al., 1990; Jiang et al., 2001; Li et al., 2010)***. Humans are not the only host for adenoviruses; different species of animals including other mammals, birds, reptiles, amphibians, and fish can also be infected with adenoviruses.

The aims of this study were to determine the prevalence of rotavirus and adenovirus infections in children and lambs with diarrhea in Najran region, and to determine the possibility of drinking water as a source of infection.

2. Material and Methods:

This study was approved by the Research and Ethics Committee of the college of Medicine, Najran University, Najran, Saudi Arabia in the year 2011.

Stool samples

The samples were collected in the period from October 2010 to April 2011, in Najran, Saudi Arabia. Ninety two stool samples were collected from children (newborn through 15 years of age) from different medical centers. On the other hand, 88 fecal samples were collected from lambs (newborn up to 3 months of age) from different farms. All the samples were collected from children and lambs suffering from diarrhea; no particular considerations were given to the sex of the patient or the season of sample collection. Each stool sample was frozen undiluted at -20°C until tested. The samples were subjected to only one thawing.

Water samples

The water samples were collected from 5 desalination stations. Viruses from 3 liters of water were concentrated in 75 ml of 50 mM glycine buffer with 3% beef extract (Oxoid) by adsorption-elution to negatively charged nitrocellulose membranes and were re-concentrated by organic flocculation in 1 ml of 0.14 N Na_2HPO_4 (pH 7) according to the method described previously ***(Abbaszadegan et al., 1999; Jiang et al., 2001)***.

Detection of the viruses by rapid kits

The presence of rotavirus and adenovirus antigens in the samples were investigated by RIDA QUICK Rotavirus/Adenovirus Combi method (R-Biopharm AG, Darmstadt, Germany), a lateral-flow immunochromatographic assay, which uses labeled monoclonal antibodies against surface antigens of rota- and adenoviruses according to the manufacturer's instructions. Briefly, each stool sample was mixed with extraction buffer before the test strip was applied to the supernatant in a separate tube. The Dipstick was steeped into a tube containing a sample for 30 sec and pulled out from a tube, and then the sample was decided whether positive or negative in 15 minutes.

Detection of the viruses by ELISA

The presence of rotavirus and adenovirus antigens in the samples were investigated by RIDA SCREN Rotavirus/ and RIDA SCREN Adenovirus (R-Biopharm AG, Darmstadt, Germany) according to the manufacturer's instructions.

RNA/DNA extraction and virus detection

RNA and DNA were extracted from 1 ml elute with the commercially available QIAamp test kit (Qiagen,Hilden, Germany), according to the manufacturer's instructions and eluted in 50 µl water.

Adenovirus DNA PCR

The primers used for amplification were 5'GCCGCAGTGGTCTTACATGCACATC-3' as a sense primer and 5'CAGCACGCCGCGGATGT-CAAAGT-3', as antisense, yielding an amplicon of 300 bp ***(Jiang et al., 2001)***. PCR was carried out using the Qiagen PCR kit (Qiagen/Westburg), according to the instructions of the supplier.

Rotavirus RNA PCR

Primers used for the detection of the virus were Beg9b GGCTTTAAAAGAGAGAATTTCCGTCT-GG 1-28 and End9b GGTCACATCAT-ACAATTCTAATCTAAG 1062-1036. The pair Beg9-End9 was used for the amplification of the full-length gene number 9 ***(Gouvea et al., 1990)***. The extracted RNA was denatured at 97°C for 5 min, and reverse transcription (RT)-PCR was carried out using the Qiagen One Step RT-PCR kit (Qiagen/Westburg) according to the instructions of the supplier.

3. Results:

Detection of rotavirus and adenovirus in children stool samples

The results of children samples showed that out of the 92 tested samples, 8 were positive for rotavirus (8.69%), and 3 were positive for adenovirus (3.26%). The results showed that 5 out of 8 (62.5%) and 2 out of 3 (66.7%) positive samples were in children less than 2 years of age in rotavirus and adenovirus respectively (Table 1). The percentage of agreement observed in both methods, RIDA QUICK Rotavirus/Adenovirus Combi method and ELISA for rotavirus and adenovirus detection was 100%. All

positive samples were confirmed positive by PCR test (data not shown).

Detection of rotavirus and adenovirus in lamb stool samples

In lambs, out of the 88 tested samples, there were 4 positive for rotavirus (4.54%) while the adenovirus could not be detected in any of the samples. The results showed also that most of the rotavirus infection, 3 out of 4 positive samples were in lambs aged less than one month (Table 2).

Detection of rotavirus and adenovirus in water samples

The result of PCR showed that the potable water samples collected from water desalination station were negative for both viruses (data not shown).

Table 1: Prevalence of rotavirus and adenovirus infections in children in Najran, Saudi Arabia

Age	Number of Samples	Rotavirus		Adenovirus	
		Positive samples	(%)	Positive samples	(%)
Less than 2 years	46	5	10.86	2	4.33
2-10 years	29	2	6.89	1	3.44
11-15 years	17	1	5.88	0	0
Total	92	8	8.69	3	3.26

Table 2: Prevalence of rotavirus and adenovirus infections in lambs in Najran, Saudi Arabia

Age	Number of Samples	Rotavirus		Adenovirus	
		Positive samples	(%)	Positive samples	(%)
Less than 1 month	48	3	6.25	0	0
1-2 month	26	1	3.84	0	0
3 month	14	0	0	0	0
Total	88	4	4.54	0	0

4. Discussion:

To investigate the prevalence of enteric viruses in Najran, two of most common enteric viruses, one RNA (Rotavirus) and one DNA (Adenovirus) were selected in this study. Although many studies have been already done on rotavirus infection in Saudi Arabia ***(Al-Bwardy et al., 1988; El Assouli et al., 1992; Al-Freihi et al., 1993; Akhter et al., 1995; Ghazi et al., 2005)***, this is the first work to be done in Najran, one of the far southwestern cities. In addition to, this is the first work to be carried out on both human and animals. In this study, the results showed that 8.7% of children and 4.5% of lambs were infected with rotavirus. Our results agreed with those results obtained in some other localities in Saudi Arabia which showed that the prevalence of rotavirus infection among children was 10% ***(Akhter et al., 1995; Ghazi et al., 2005)***. Further, our results showed that rotavirus infection are much more prevalent among infants. These results are comparable to those from other localities in Saudi Arabia which showed the occurrence of rotavirus at a rate of approximately 40% among the infants hospitalized for acute diarrhea ***(Al-Bwardy et al., 1988; El Assouli et al., 1992)***.The variation between our results and the previous ones may be due to the difference in diagnostic tools used in both works.

The finding that most rotavirus infection occurs among children aged less than 2 years is also consistent in most studies from other parts of the world ***(Parashar et al., 2003; Verheyen et al., 2009)***. On the other hand, we have detected the virus in lambs with percent of 4.5% which coincide with infection of lambs with rotavirus in other parts of the world ***(Theil, 1990; Russell and Benk, 1999; Fong and Lipp 2005)***. In agreement with results obtained in other parts of the world, our results showed that 3.3% of infection occurs in children. In industrialized countries, the incidence varies from 1 to 8%, whereas in developing countries varies from 2 to 31% ***(Weitzel et al., 2007)***.

Although, previous studies indicated the presence of rotavirus and adenovirus in water ***(Puig et al., 1994; Pusch et al., 2005; Verheyen et al., 2009)***, our results could not detect both viruses in the tested potable water samples. One possible causes of infection of human in Najran could be attributed to the non potable water which is used in household purpose. The population residing in Najran area uses multiple types of water sources for drinking and other household purpose, such as cleaning and bathing. The problem may reside in using the non-potable water for washing of fresh uncooked food such as vegetables and fruits, which may explain the infection of humans with these viruses. The sheep probably plays a role in the interspecies transmission responsible for the introduction of rotavirus strains into the human population. The phylogenetic analyses

confirmed that a common origin for the human P [14] strains and those of the even-toed ungulates belonging to the mammalian order Artiodactyla ***(Matthijnssens et al., 2009)***. Molecular detection techniques based on host specificity of viral pathogens in environmental samples would allow the determination of the sources of contaminants and improve surveillance for public health ***(Metcalf et al., 1995)***.

Rida Quick rotavirus/adenovirus Combi test achieved 100% sensitivity in comparison to ELISA. This result allows the use of Rida Quick rotavirus/adenovirus Combi test as easy, fast, sensitive and non expensive method for diagnosis of rotavirus and adenovirus in developing countries. This is the first work to study rotavirus infection and adenovirus infection in this region. Further studies are needed firstly, to investigate theses viruses in non potable water. Secondly, to characterize the viruses isolated from human and animal.

ACKNOWLEDGMENT

The authors thank the dean of scientific research, Najran University, Najran, Saudi Arabia for sponsoring this study. We also thank Dr. Nikolaus Osterrieder for his kind support of this work.

Corresponding author
Abuelyazeed A. Elsheikh,
Department of Applied Medical Sciences, Community College, Najran University, Najran, Box: 1988, Saudi Arabia.
Permanent Address: Department of Virology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, 44511, Egypt
E-mail address: eaa000@yahoo.com

References:

1. Abbaszadegan, M., P. Stewart, and M. LeChevallier., 1999. A strategy for detection of viruses in groundwater by PCR. Appl Environ Microbiol., 65: 444-449.
2. Akhter, J., S. al-Hajjar, S. Myint, and S. M. Qadri., 1995. Viral contamination of environmental surfaces on a general paediatric ward and playroom in a major referral centre in Riyadh. Eur J Epidemiol., 11: 587-590.
3. Al-Bwardy, M. A., S. Ramia, A. R. al-Frayh, A. H. Chagla, A. A. al-Omair, M. A. el-Hazmi, A. Lambourne, H. Bahakim, and H. Salman., 1988. Bacterial, parasitic and viral enteropathogens associated with diarrhoea in Saudi children. Ann Trop Paediatr., 8: 26-30.
4. Al-Freihi, H., K. Twum-Danso, M. Sohaibani, H. Bella, M. el-Mouzan, and K. Sama., 1993. The microbiology of acute diarrhoeal disease in the eastern province of Saudi Arabia. East Afr Med J., 70: 267-269.
5. Allard, A., B. Albinsson, and G. Wadell, 1992. Detection of adenoviruses in stools from healthy persons and patients with diarrhea by two-step polymerase chain reaction. J Med Virol., 37: 149-157.
6. Bosch, A., 1998. Human enteric viruses in the water environment: a minireview. Int Microbiol., 1: 191-196.
7. Crabtree, K. D., C. P. Gerba, J. B. Rose, and C. N. Haas, 1997. Waterborne adenovirus: A risk assessment. Water Science and Technology, 35: 1-6.
8. Cruz, J. R., P. Caceres, F. Cano, J. Flores, A. Bartlett, and B. Torun, 1990. Adenovirus types 40 and 41 and rotaviruses associated with diarrhea in children from Guatemala. J Clin Microbiol., 28: 1780-1784.
9. El Assouli, S. M., Z. M. Banjar, K. A. Mohammed, and F.T. Zamakhchari, 1992. Rotavirus infection in children in Saudi Arabia. Am J Trop Med Hyg., 46: 272-277.
10. Estes, M. and A. Kapikian, 2007. Rotaviruses, In D. M. Knipe, P. M. Howley, D. E. Griffin, R. A. Lamb, M. A. Martin, B. Roizman, and S. E. Straus (ed.), Fields virology, 5 edn: Lippincott, Williams and Wilkins, Philadelphia, PA. 1917-1974.
11. Fong, T. T. and E. K. Lipp, 2005. Enteric viruses of humans and animals in aquatic environments: health risks, detection, and potential water quality assessment tools. Microbiol Mol Biol Rev., **69:** 357-371.
12. Ghazi, H. O., M. A. Khan, A. M. Telmesani, B. Idress, and M. F. Mahomed, 2005. Rotavirus infection in infants and young children in Makkah, Saudi Arabia. J Pak Med Assoc., 55: 231-234.
13. Gouvea, V., R. I. Glass, P. Woods, K. Taniguchi, H. F. Clark, B. Forrester, and Z. Y. Fang, 1990. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J Clin Microbiol., 28: 276-282.
14. Griffin, D. W., K. A. Donaldson, J. H. Paul, and J. B. Rose, 2003. Pathogenic human viruses in coastal waters. Clin Microbiol Rev., 16: 129-143.
15. Horwitz, M. 1996. Adenoviruses, In: Fields BN, Knipe DM, Howley PM, eds. Virology, 3 edn: Philadelphia: Lippincott-Raven. p. 2149-2171.
16. Hunter, P. R., 1997. Waterborne Disease: Epidemiology and Ecology. Wiley, Chichester, United Kingdom.

17. Ishibashi, M. and H. Yasue, 1984. Adenoviruses of animals, In H. S. Ginsberg (ed.), The adenoviruses: Plenum Press, New York, N.Y. p. 497-562.
18. Jiang, S. C., 2006. Human adenoviruses in water: occurrence and health implications: a critical review. Environ Sci Technol., 40: 7132-7140.
19. Jiang, S., R. Noble, and W. Chu, 2001. Human adenoviruses and coliphages in urban runoff-impacted coastal waters of Southern California. Appl Environ Microbiol., 67: 179-184.
20. Kheyami, A. M., N. A. Cunliffe, and C.A. Hart, 2006. Rotavirus infection in Saudi Arabia. Ann of Saudi Med., 26: 184-191.
21. Lee, S. H. and S. J. Kim, 2002. Detection of infectious enteroviruses and adenoviruses in tap water in urban areas in Korea. Water Res., 36: 248-256.
22. Li, D., M. He, and S. C. Jiang, 2010. Detection of infectious adenoviruses in environmental waters by fluorescence-activated cell sorting assay. Appl Environ Microbiol., 76: 1442-1448.
23. Matthijnssens, J., C. A. Potgieter, M. Ciarlet, V. Parreno, V. Martella, K. Banyai, L. Garaicoechea, E. A. Palombo, L. Novo, M. Zeller, S. Arista, G. Gerna, M. Rahman, and M. Van Ranst, 2009. Are human P[14] rotavirus strains the result of interspecies transmissions from sheep or other ungulates that belong to the mammalian order Artiodactyla? J Virol., 83: 2917-2929.
24. Metcalf, T. G., J. L. Melnick, and M. K. Estes, 1995. Environmental virology: from detection of virus in sewage and water by isolation to identification by molecular biology--a trip of over 50 years. Annu Rev Microbiol., 49: 461-487.
25. Parashar, U. D., E. G. Hummelman, J. S. Bresee, M. A. Miller, and R. I. Glass, 2003. Global illness and deaths caused by rotavirus disease in children. Emerg Infect Dis., 9: 565-572.
26. Puig, M., J. Jofre, F. Lucena, A. Allard, G. Wadell, and R. Girones, 1994. Detection of adenoviruses and enteroviruses in polluted waters by nested PCR amplification. Appl Environ Microbiol., 60: 2963-2670.
27. Pusch, D., D. Y. Oh, S. Wolf, R. Dumke, U. Schroter-Bobsin, M. Hohne, I. Roske, and E. Schreier, 2005. Detection of enteric viruses and bacterial indicators in German environmental waters. Arch Virol., 150: 929-947.
28. Russell, W. C. and M. Benk, 1999. Animal viruses, In A. Granoff and R. G. Webster (ed.), Encyclopedia of virology, 2 edn: Academic Press, London, United Kingdom. p. 14-21.
29. Theil, K. W., 1990. *Group A rotaviruses.* In: Viral diarrheas of man and animals: CRC Press, Boca Raton, FL. p. 35-72.
30. Verheyen, J., M. Timmen-Wego, R. Laudien, I. Boussaad, S. Sen, A. Koc, A. Uesbeck, F. Mazou, and H. Pfister, 2009. Detection of adenoviruses and rotaviruses in drinking water sources used in rural areas of Benin, West Africa. Appl Environ Microbiol., 75: 2798-2801.
31. Weitzel, T., K. Reither, F.P. Mockenhaupt, K. Stark, R. Ignatius, E. Saad, A. Seidu-Korkor, U. Bienzle, and E. Schreier, 2007. Field evaluation of a rota- and adenovirus immunochromato-graphic assay using stool samples from children with acute diarrhea in Ghana. J Clin Microbiol., 45: 2695-2697.
32. Wilhelmi, I., E. Roman, and A. Sanchez-Fauquier, 2003. Viruses causing gastroenteritis. Clin Microbiol Infect., 9: 247-262.

2/2/2012