



sky fox

ISSN: 2454-6127



## REVIEW ARTICLE

# Human Norovirus Infection: Identification, Epidemics and Treatment

Başak Gökçe ÇÖL<sup>1</sup>, Seydi YIKMIŞ<sup>\*2</sup>

<sup>1</sup>Istanbul Gelişim University, School of Health Sciences, Department of Nutrition and Dietetics, Istanbul, Turkey

<sup>2</sup>Namık Kemal University, School of Health Sciences, Department of Nutrition and Dietetics, Tekirdağ, Turkey

\*Author to whom correspondence should be addressed/E-Mail: [syikmis@hotmail.com](mailto:syikmis@hotmail.com)

Received: Mar 2017 / Accepted: Mar 2017/ Published: Mar 2017

**ABSTRACT:** Human Noroviruses (HuNoVs) are important enteric pathogens, which affect the stomach and intestines, leading to gastroenteritis or more commonly called the "stomach flu" or "winter vomiting bug". HuNoVs are mainly transmitted by the fecal-oral route, either by directly infected person-to-person contact or directly via contaminated foods, water and surface areas. The virus is highly contagious as 10-100 virus particles are sufficient to cause diseases. HuNoVs can spread easily and cause prolonged outbreaks. This is due to their environmental persistence, high infectivity, being resistance to disinfection and difficulty in preventing transmission. HuNoVs are the most common causative agent leading to acute gastroenteritis among infectious diseases worldwide and poses a serious public health problem, especially among children being the most susceptible. In developing countries, the highest cost of medical care after respiratory infections is listed for acute gastroenteritis. In this study, Norovirus outbreaks, precautions, its identification and struggles were informed and some suggestions were made about this case.

**Keywords:** Human Noroviruses, gastroenteritis, outbreak

## INTRODUCTION

HuNoVs are non-enveloped, a single-stranded RNA viruses, belong to the genus Norovirus, in the Caliciviridae family. Norovirus strains have been divided into seven genogroups (GI-GVII), which can be further classified into at least thirty genotypes. GI, GII, and GIV infect humans whereas GII, GIV, GV, GVI and GVII NoVs (Noroviruses) have been determined in animals (da Silva Poló *et al.*, 2017).

In recent years, viruses are defined as important factors in foodborne diseases. Although viruses cannot reproduce in food and environmental surfaces, they serve as vectors which allow them to easily penetrate humans. Primary factors in the transmission of viral agents are: contamination of drinking water with sewage water, use of underground waters mixed with sewage water for vegetable growing, washing of raw vegetables with contaminated water, preparation of food by infected personnel, and catching of shellfish from contaminated areas. Additionally, secondary contamination factors are contamination of foodstuffs during processing, storage, distribution or final preparation (Dreyfuss, 2009; A. Thornton, Jennings-Conklin, & McCormick, 2004).

One of the foodborne viruses is HuNoV (Human norovirus), which causes digestive system infections in humans. HuNoVs are accepted as the worldwide reason for 60-80% of acute gastroenteritis outbreaks which affect the individuals of all age groups (R L Fankhauser *et al.*, 1998; B. A. Lopman *et al.*, 2003). The infections caused by HuNoVs are mainly transmitted by the faecal-oral route. The main factors in the spread of HuNoVs in the population and their outbreaks are:

Low infective dose (10-100 viral particles)

High viral load (up to 10<sup>12</sup> genomic copies) in the vomit and feces of infected individuals

Resistance of the agent to environmental conditions (R L Atmar *et al.*, 2008)

HuNoV is one of the most common causes of outbreaks in environments where individuals coexist such as schools, passenger ships, restaurants, hospitals, nursing homes, camps and dormitories. HuNoV outbreaks are associated with contamination by contaminated food makers to a considerable extent. On the other hand, although the first source is contaminant food or water as well as the spreading fecal-oral route or aerosol exposure, the contamination directly from the person through the surface / bodies of contaminants also plays a major role.

Laboratory studies with HuNoV have shown that virus can be easily transferred between food, food contact surfaces, hands and the environment. Workers working in the food industry often go back to work and continue to work in the same department, even after treatment, after complaints of gastroenteritis. However, in many cases, even if there are no clinical signs of disease, still the virus spread continues. For this reason, it is important to prevent the transfer of virus from infected food personnel. It has been shown that HuNoV is one of the most important factors of acute gastroenteritis outbreaks and if it is not prevented it could spread to large populations in a short time and threaten public health (R L Fankhauser *et al.*, 1998; Widdowson *et al.*, 2005).

In HuNoV infections, mortality is not high, but it is dangerous in children and in immunocompromised individuals. Particularly in places where people are collectively present (such as hospitals, nurseries, ships, prisons, hospitals, restaurants) it facilitates the emergence and spread of the disease. HuNoVs which resistant to environmental factors (cold and 60 ° C temperature) are an important source of these viruses for spreading and spreading viruses by protecting their environment for 3-4 weeks on their surface (Kageyama *et al.*, 2003). The main causes of the outbreaks are compounded by the fact that the post-disease agent can be found in the stool for an extended period and also that the contaminated source and network waters spread rapidly by affecting the large masses.

In the health sector, EPA (Environmental Protection Agency) approved HuNoV has disinfectants that are approved for use in inactivation. Not all of these disinfectants are suitable for use in the food sector. Additionally, there are no disinfectant standards currently in use in the food sector against HuNoV viruses. Based on the available literature, no studies have been found on all the agents in the mediating role in the emergence of outbreaks in the epidemiologically relevant epidemiologic context of the onset of an epidemic infection of HuNoV in the world. In this study, we tried to give detailed information about HuNoVs.

## GENERAL INFORMATION

### 1.1. History of NoVs

The Norwalk virus was first described in 1968 as an effect of viral gastroenteritis in an electron microscope after four years of stool specimens in acute gastroenteritis cases in a Norwalk primary school in Ohio, USA (Jiang, Matson, Cubitt, & Estes, 1996; Kapikian *et al.*, 1972). In later epidemics, they were named after the epidemic that they had as Montgomery County, Snow Mountain, Mexico, Hawaii, Jena, Taunton, and Toronto viruses (Hardy, Kramer, Treanor, & Estes, 1997; Jiang *et al.*, 1995; Lew, Petric, *et al.*, 1994; Lew, Kapikian, Valdesuso, & Green, 1994; B. Lopman, Zambon, & Brown, 2008). After a massive outbreak in Toronto, it had been reported that this virus is the second most important viral gastroenteritis that affect children. Later, it was described as "Norwalk-like virus" with the name of the place where the first epidemic occurred, further simplified as "Norovirus" by the International Virus Taxonomy (Robert L. Atmar & Estes, 2006; Kapikian *et al.*, 1972).

### 1.2. Etiological Features of HuNoV

Even seven genotypes of NoV exist, the most common NoV (G II and GIV) is a major cause of outbreaks worldwide (Ozawa, Oka, Takeda, & Hansman, 2007). HuNoV has a more durable structure than enveloped viruses, it can withstand acid, chloroform, ether, chlorine (<10 ppm), alcohol (70% partially inactivated), cold and heat up to 60 ° C. They are more resistant to disinfectants than enteroviruses (R L Atmar & Estes, 2001; Koch, Schneider, Stark, & Schreier, 2006; Percival, Yates, Williams, Chalmers, & Gray, 2004). RNA, the genome of HuNoV consists of positive polarity, single-chain and 7400-7,700 nucleotides. The genomes with these features serve as mRNA. Whenever the virus enter the target-cell, it bounds to cell ribosomes and protein translation occurs. It encodes three reading regions encoding the genomic protein; ORF1 encodes non-structural proteins such as RNA-dependent RNA polymerase and helicase, while ORF2 encodes major capsid protein (VP1) and ORF3 encodes minor capsid protein (VP2) (Clarke & Lambden, 2002).

### 1.3. Outbreaks of HuNoV in the World

Norovirus is highly contagious with about 10-100 virus particles sufficient for infection development (Ustaçelebi, Ş., Abacıoğlu, H., Badur, 2004). Noroviruses mainly go through the fecal-oral route and enter the human body through the mouth and pass through the small intestine without being affected by the stomach pH. Viral replication occurs in the small intestinal mucosal epithelium, resulting in damage to the small intestine enterocytes due to reproduction and flattening in the villi, with clinical manifestations occurring after 24 hours of incubation (Patel *et al.*, 2009; A. C. Thornton, Jennings-Conklin, & McCormick, 2004). Noroviruses can be transmitted by direct contact with infected individuals or foods, water and surfaces contaminated by them (Verhoef *et al.*, 2010). Infected individuals begin to spread the disease from the time the symptoms develop and may continue contaminating for two weeks after recovery. The incubation period of the virus is approximately 1-2 days. Clinical findings usually last 12-72 hours, but the virus can be removed by the patient's feces for 2-3 weeks (Teunis *et al.*, 2008).

Symptoms such as nausea, vomiting and watery diarrhea are seen. Typical symptoms such as diarrhea, vomiting, abdominal pain, cramps, weakness, low fever usually heal spontaneously in a short period. In some patients, only vomiting can be seen and the disease has been referred to as 'winter vomiting disease' (Iturriza-Gómara & Lopman, 2014; Jiang *et al.*, 1995; Koo, Ajami, Atmar, & DuPont, 2010; Said, Perl, & Sears, 2008; Treanor & Dolin, 2000). In recent years, norovirus-induced infections are also called gastric flu (M Koopmans *et al.*, 2009). In infants, elderly, immunosuppressed individuals, necrotizing enterocolitis and pediatric oncologic patients, infections are more severe but the disease typically tends to be mild in nature with death being rare (Patel *et al.*, 2009).

The outbreaks of HuNoV can be seen all year round, but are more frequent in winter months. HuNoVs are responsible for more than 90% of viral gastroenteritis worldwide and about 50% of different etiologic acute gastroenteritis. According to the Centers for Disease Control (CDC) from 1996 to 2000; of the 348 epidemics that occurred: 39% food, 12% human-to-human transmission, 3%

water, and 18% of them cannot be detected (Parashar *et al.*, 2001). In a study conducted in the USA between 2000 and 2004, it was determined that contagion occurs mostly because of human to human transmission, secondly due to contaminated foods (Blanton *et al.*, 2006).

While it is reported that HuNoV-originated food outbreaks in the US are 15,000 hospital cases per year and 1,500 deaths, It is estimated that more than 600,000 HuNoV-infected cases are reported each year in the UK, especially in the winter months. In the surveillance studies of acute gastroenteritis (AGE) outbreaks in the US and European countries between 1995 and 2000, it is found that 43% to 95% of outbreaks were caused by HuNoVs (Scallan, Griffin, Angulo, Tauxe, & Hoekstra, 2011).

Surveillance studies of acute gastroenteritis (AGE) outbreaks in the US and European countries between 1995 and 2000 found that 43% to 95% of outbreaks were caused by HuNoVs. People are rarely receiving medical help because of the illness, which is mostly mildly observed, and only part of the HuNoV cases around the world are officially reported.

For a disease that is mostly mild, people rarely receive medical treatment for it, and therefore only some of the HuNoV cases in the world are reported to the public. The rates of HuNoV in AGE outbreaks seen worldwide in 1996-2000 are over 95% in the US, UK, Denmark, Sweden, Finland, the Netherlands and France, 84% in Holland, 57% in Spain and 43% (B. A. Lopman *et al.*, 2003). In 2005, a case of Noroviruses (GGII) in Denmark was found to have originated from frozen raspberries imported from Poland. There was a Norovirus outbreak affecting 652 people in 6 hospitals (31.4%) and 11 nursing homes (35.2%) in the Catalan region of Spain, with 94.1% of people spreading the virus and 5.9% of those infected by the food (Godoy *et al.*, n.d.).

In Hong Kong, more than 60% of the AGE infections seen in nursing homes in 2001-2006 were identified as HuNoVs (Ho, Cheng, Lau, Wong, & Lim, 2007). Between 2001 and 2006, 7637 HuNoV outbreaks were detected in 13 European countries and in these outbreaks, HuNoV was the most identifiable genotype of GG II.4 type (Kroneman *et al.*, 2008).

There are very few studies in Turkey compared to the studies conducted in different regions in the world. The work done is on HuNoV from clinical samples and food; surface and hands are not directed towards HuNoV detection. The main reason for this is that until 2008, no studies on epidemic HuNoV outbreaks have been made because of the inadequacy of surveillance systems for viral AGEs in the country.

Among the prominent studies in Turkey, HuNoV was diagnosed by RT-PCR in 15 (17%) of the 88 children with diarrhea at Afyon Kocatepe University Medical Faculty Pediatrics Service in 2006-2007 in relation to the diagnosis of sporadic HuNoVs (Altindis *et al.*, 2009). In 2008, the outbreak of NoV in Aksaray was determined. This epidemic occurred when 25 students who were studying in a high school went to the hospital with complaints of nausea, vomiting, abdominal pain and diarrhea. In addition, similar incidents were observed simultaneously in the districts of Gülağaç, Ortaköy and Sarıyahşi and in neighboring provinces Şereflikoçhisar / Ankara and Konya (Uyar, Carhan, Ozkaya, & Ertek, 2008). GI and GII were found to be the most common genogroups among NoVs diagnosed by PCR in stool specimens examined. In a study conducted to reveal the frequency of HuNoV presence in Turkey, HuNoV GI and GII species in tomatoes, parsley, green onions, lettuce, mixed salad and bulgur prepared foods, were the pathogens reported in humans (Yılmaz *et al.*, 2011).

Uyar and colleagues (2008) assessed a total of 50 stool specimens taken from the cases of "diarrhea and nausea-vomiting" in Aksaray, Şereflikoçhisar, Kırşehir and Adana, where possible bacterial, viral and parasitic factors could not be detected in terms of HuNoV. ELISA and RT-PCR methods were used for HuNoV laboratory diagnosis, where 26% (13/50) of the stool specimens were positive for antigen, and 33% (13/40) were positive for nucleic acid.

#### 1.4. Laboratory Diagnosis

The laboratory diagnosis is especially important for the detection of outbreaks. Immunoelectron microscopy was used to show some viruses that could not be produced in cultures in stool specimens, but due to the inadequacy of routine use, new methods had to be developed over time. Today, methods commonly used in clinical diagnosis are mainly based on showing the nucleic acids or antigenic structures of the virus (Kele, Lengyel, & Deak, 2011; A. Richards *et al.*, 2003).

ELISA is a valuable method that it is highly sensitive and it can be used to examine many samples in a short time. The antigen can be searched in feces by ELISA for a rapid diagnosis of HuNoV (Rabenau *et al.*, 2003).

Electron microscopy, ELISA and molecular methods are used in the diagnosis of gastroenteritis caused by HuNoV (Blanton *et al.*, 2006; Costantini, Loisy, Joens, Le Guyader, & Saif, 2006; Rabenau *et al.*, 2003).

RT-PCR can be used for both different clinical samples as well as for food, water and other environmental sample studies (Koo *et al.*, 2010). With RT-qPCR, it is also possible to quantitatively estimate the viral load. Molecular detection of foodborne pathogens is very difficult due to the low amount of virus particles and the presence of substances that cause inhibition (Estes, Prasad, & Atmar, 2006). Many features of HuNoVs are not well understood because of the inability to cultivate and ineffective use of test animals until now. The data related to HuNoV infectivity is limited due to the difficulty of carrying out voluntary human experiments. (Dolin *et al.*, 1972) Because no animal or cell culture models were available for HuNoVs; due to this situation its surrogates ; Feline calicivirus (FCV),

murine norovirus (MNV), and colifaj MS2 have used more frequently in inactivation studies (Baert, Uyttendaele, Van Coillie, & Debevere, 2008; Duizer *et al.*, 2004).

The recently-described Tulane virus (TV) is a typical calicivirus. The in vitro cultured Tulane Virus (TV) recognizes human tissue-blood group antigens (HBGA). The TV known as the monkey calicivirus has been isolated from the National Primate Research Center's rhesus monkey faeces. In vitro, rhesus monkey replicates in kidney cells and causes typical cytopathic effects in cells. Unlike MNV and FCV, TV recognizes Type B HBGA receptors for infecting (Farkas *et al.*, 2010).

In 2017, Scientists at Baylor College of Medicine have, for the first time grown NoV in human intestinal epithelial cells. This will allow for promising developments in diagnosis, prevention and treatment (Graciela Gutierrez, n.d.).

### 1.5. Treatment and Prevention

Norovirus gastroenteritis usually heals spontaneously without requiring any treatment. Difficulties about cell culture studies has been a barrier to the development of antiviral drugs for HuNoV. Until now there is no specific licensed vaccination for HuNOV. Mechanisms of immunity to HuNoVs are unclear. It appears that immunity may be strain-specific and lasts only a few months; therefore, given the genetic variability of noroviruses, individuals are likely to be repeatedly infected throughout their lifetimes. The main principle in treatment is the prevention of dehydration by replacement of isotonic fluids. Approximately 10% of people with Norovirus gastroenteritis may require oral or intravenous fluid therapy for dehydration. In addition, analgesics, antiemetics and symptomatic drug treatment are applied to clinical findings such as muscle aches, headache and vomiting (Hall *et al.*, 2011; Kiraz, Samastı, & Aygün, 2011; Verhoef *et al.*, 2010).

Antiviral agents targeting the binding of carbohydrate receptors in norovirus enterocytes are being studied for treatment (Parra *et al.*, 2012). Most of the European countries have established data systems that evaluate / inform health and epidemic (Reintjes, Thelen, Reiche, & Csohan, 2007).

It is difficult to control the outbreak of norovirus infection by food, water, personal contact and environmental surfaces. Since outbreaks are usually caused by water and food, it is important to protect these sources from being contaminated. Because the virus is highly resistant to environmental conditions, foodstuffs must be subjected to adequate heat treatment and sewage contact should be prevented to the water resources to be used in the production of food. Foodborne contamination is associated with a greater risk of contamination of all kinds of food, especially marine products such as mussels, oysters, fresh vegetables and fruits (Gallimore *et al.*, 2004; Patel *et al.*, 2009; Westrell *et al.*, 2010).

No	Precautions
1	Hands should be washed often
2	Contaminant surfaces should be completely cleaned and disinfected if there are sick persons in the environment.
3	Contaminant surfaces should be completely cleaned and disinfected if there are sick persons in the environment.
4	Sodium hypochlorite, the most effective disinfectant in surface disinfection should be used.
5	Clothes should be changed and washed immediately after contamination from vomit or feces.
6	Avoiding direct contact with infected individuals, and contaminated vectors such as food, water or other objects.
7	Patients should be allowed to return to work after the symptoms have completely disappeared.
8	Patients should be informed that they will be the source of virus outbreaks for a long time after healing.

**Table 1:** General precautions to be taken to prevent Norovirus infections (Uyar *et al.*, 2008).

If contamination is detected in water sources, chlorination must be done at a high level ( $\geq 10$  mg / l) for Noroviruses. Contaminated areas should be cleaned with disinfectants, 10% sodium hypochlorite with germicides and contaminant bed covers should be washed with detergents containing bleach and at least 70 ° C (Öztürk, 2008). Contaminated areas should be cleaned with hypochlorite-containing disinfectants (10% sodium hypochlorite solution) or appropriate germicides (Rebecca L. Fankhauser *et al.*, 2002).

### 1.6. HuNoV Durability and Inactivation in Environmental Surfaces and Hands

Detection of the agent from environmental surfaces during gastroenteritis outbreaks in HuNoV indicates that HuNoV may be infectively found on environmental surfaces for extended periods of time. HuNoV is often infected with the fecal contents of infected persons and the surrounding surfaces and clothing through hands and the infected foods. Studies on the presence of HuNoV in the hands were mainly carried out using viruses (MNV, FCV etc.) or similar viruses such as Hepatitis A (Todd, Greig, Bartleson, & Michaels, 2009).

In these studies, it has been shown that enteric viruses can survive for several hours in human hands. In the investigation of outbreaks, the swine from the patient's food handler showed that the detection of HuNoV RNA showed the virus remained long-term in the hands (Boxman *et al.*, 2009). In 2009, Liu and his colleagues showed in their study that HuNoV RNA can still survive in the hands for more than two-hours.

### 1.7. Inactivation Methods of HuNoVs on Surfaces and Hands

As long as there are HuNoVs that are resistant to the environment, HuNoVs will cause major problems in both the food chain and the healthcare industry (B. Lopman *et al.*, 2012). In general, measures for food hygiene are designed to control the reproduction of harmful bacteria in food production (such as protecting the cold chain) (Gary P Richards, 2012). HuNoVs cannot reproduce in the human body for a long time and can maintain their durability in environmental conditions, so cool and humid conditions keep them alive instead of inactivating them. As a result, there is a need for food hygiene solutions specifically designed for the inactivation of enteric viruses, as well as for the control of bacteria in the prevention of foodborne outbreaks. In the health sector, strict hygiene measures are needed to prevent and control the spread of HuNoVs. Several chemical and physical inactivation methods have been developed for the control of infections caused by HuNoV. Chlorine, is the most frequently used disinfectant because of its ease of application, reliability, cost effectiveness, residual biocide effect and superior effect against bacteria and viruses. Ethanol, sodium bicarbonate, ozone and quaternary ammonium compounds are some of the chemicals used for the inactivation of FCV and MNV (Rockx *et al.*, 2002; Wobus *et al.*, 2004). However, there is a need for more precise information on the effectiveness of HuNoV inactivation methods.

According to European disinfection standards (Sickbertbennett *et al.*, 2005), inactivation of viral contaminants requires quantitative suspension and environmental surface testing at a maximum of 60 min above 20 ° C and at least 4 log reduction in all test groups. Chemical and physical disinfection methods including sodium hypochlorite, heat and UV methods are used against HuNoV and other enteric viruses (Marion Koopmans & Duizer, 2004). HuNoV infectivity cannot be done in the laboratory, except for voluntary work. Inactivation assays are based on genomic detection methods using resistant HuNoV virus-like particles (VLP) containing viruses or RNA representing HuNoV (G P Richards, 1999).

Chemical disinfection is the most common approach to break the chain of contact with food and other environmental surfaces of HuNoVs. Centers for Disease Control and Prevention recommend the use of a sodium hypochlorite solution in the disinfection of environmental surfaces that are potentially contaminated with HuNoV (Maccannell *et al.*, 2011). However, although sodium hypochlorite is effective against HuNoV-representing viruses, HuNoV RNA cannot be completely destroyed (Barker, Vipond, & Bloomfield, 2004; Sickbertbennett *et al.*, 2005). There are various contradictions in the efficacy of alcohols against HuNoV. It has been shown that 55-60% ethanol solution inactivates MNV at 6 log<sub>10</sub> levels over 5 minutes (Magulski *et al.*, 2009). In 2010 Girard and colleagues showed that 2-1- (Butoxy) -prapronol and ethoxyl alcohols had no effect on HuNoV and MNV.

The most important method of preventing the spread of HuNoV infections through the hand and controlling the transmission of viruses, is through proper hand hygiene. Ethanol-based hand sanitizers are frequently used for the inactivation of bacterial and respiratory viruses (Maccannell *et al.*, 2011). However, the effectiveness of various ethanol-based chemistries on HuNoVs is still unclear. It has been observed by the FDA in a 2010, food safety survey, that washing hands at home using water and soap before food preparation has reduced the incidence of foodborne illnesses (Ali, Verrill, & Zhang, 2014).

Outbreaks from HuNoV in hospitals and other health care facilities can sometimes last for months (Said, Perl, Sears, & Sears, 2008). Infection may be more severe in inpatients leading to death than healthy individuals. Young children and geriatrics have a higher incidence of infection.

Foods can be contaminated with fecal contamination by non-hygienic applications from food personnel. The food staff can easily infect food and surrounding surfaces from their hands. Especially in the food production line, there is a high possibility that contaminants from surfaces such as refrigerators, door handles and work benches, are found in foods. It is not a surprise that foodborne poisoning and outbreaks are caused by sandwiches and salads that are processed by hand or that are not re-heat treated after preparation. Increasing consumption of these types of ready-to-eat food in modern life increases the rate of food-borne HuNoV infections that the virus uses as a means of such food.

When assessed from the food industry point of view, avoiding secondary contaminations caused by food workers is an important preventive measure to be taken in the fight against this disease. As well as the prevention of contamination sources, another point to be

considered to prevent a possible contamination in foods is some physical (heat treatment, UV, etc.) or chemical (chlorine, ozone, etc.) applications carried out by producers.

Physical or chemical applications by producers as well as the prevention of contamination sources, another point to be considered to prevent a possible contamination in foods is the physical (heat treatment, UV, etc.) or chemical (chlorine, ozone, etc.) applications by producers

As a result, HuNoVs have an important place in foodborne illnesses. Due to the high infectious character, HuNoV may be encountered on peripheral surfaces, hands and any part of the food chain, as it passes through human to human easily. It is possible that HuNoV is encountered on peripheral surfaces, hands and any step of the food chain. It is an important research topic today to determine the effects of chemical methods such chlorine, ozone, sodium bicarbonate, heat treatments like high pressure applications and freezing, on the inactivation of HuNoVs in different environments to ensure the reliability of food in terms of viruses (Elmnasser *et al.*, 2007).

## CONCLUSION

Rapid laboratory diagnosis is important due to the rapid spread of Norovirus infection and its impact on large communities in a short duration, significant economic and labor loss, and deaths. Today, scientists are focusing on the development of faster and more accurate methods to be used in virus detection studies. It is difficult to control Norovirus-associated outbreaks as Noroviruses are easily transmitted through food, water, personal contact and environmental surfaces. Protective health services and scientific applications should be carried out on time and with an effective manner.

As a result, the general health rules must always be applied to infectious diseases and the level of sanitation and hygiene should be increased. Additionally, epidemiological studies to determine the source of the epidemic should be initiated from the first day and the cause of the epidemic should be identified as soon as possible so that scientific and technical measures can be taken in the epidemic response process.

## REFERENCES

1. Ali, M. M., Verrill, L., & Zhang, Y. (2014). Self-Reported Hand Washing Behaviors and Foodborne Illness: A Propensity Score Matching Approach. *Journal of Food Protection*, 77(3), 352–358. <https://doi.org/10.4315/0362-028X.JFP-13-286>
2. Altindis, M., Bányai, K., Kalayci, R., Gulamber, C., Koken, R., Yoldas, Y., Martella, V. (2009). Frequency of norovirus in stool samples from hospitalized children due to acute gastroenteritis in Anatolia, Turkey, 2006–2007. *Scandinavian Journal of Infectious Diseases*, 41(9), 685–688. <https://doi.org/10.1080/00365540903071342>
3. Atmar, R. L., & Estes, M. K. (2001). Diagnosis of noncultivable gastroenteritis viruses, the human caliciviruses. *Clinical Microbiology Reviews*, 14(1), 15–37. <https://doi.org/10.1128/CMR.14.1.15-37.2001>
4. Atmar, R. L., & Estes, M. K. (2006). The Epidemiologic and Clinical Importance of Norovirus Infection. *Gastroenterology Clinics of North America*, 35(2), 275–290. <https://doi.org/10.1016/j.gtc.2006.03.001>
5. Atmar, R. L., Opekun, A. R., Gilger, M. A., Estes, M. K., Crawford, S. E., Neill, F. H., & Graham, D. Y. (2008). Norwalk virus shedding after experimental human infection. *Emerg Infect Dis*, 14(10), 1553–1557. <https://doi.org/10.3201/eid1410.080117>
6. Baert, L., Uyttendaele, M., Van Coillie, E., & Debevere, J. (2008). The reduction of murine norovirus 1, *B. fragilis* HSP40 infecting phage B40-8 and *E. coli* after a mild thermal pasteurization process of raspberry puree. *Food Microbiology*, 25(7), 871–874. <https://doi.org/10.1016/j.fm.2008.06.002>
7. Barker, J., Vipond, I. B., & Bloomfield, S. F. (2004). Effects of cleaning and disinfection in reducing the spread of Norovirus contamination via environmental surfaces. *Journal of Hospital Infection*, 58(1), 42–49. <https://doi.org/10.1016/j.jhin.2004.04.021>
8. Blanton, L. H., Adams, S. M., Beard, R. S., Wei, G., Bulens, S. N., Widdowson, M., ... Monroe, S. S. (2006). Molecular and Epidemiologic Trends of Caliciviruses Associated with Outbreaks of Acute Gastroenteritis in the United States, 2000–2004. *The Journal of Infectious Diseases*, 193(3), 413–421. <https://doi.org/10.1086/499315>
9. Boxman, I., Dijkman, R., Verhoef, L., Maat, A., van Dijk, G., Vennema, H., & Koopmans, M. (2009). Norovirus on swabs taken from hands illustrate route of transmission: a case study. *Journal of Food Protection*, 72(8), 1753–5. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/19722414>
10. Clarke, I. N., & Lambden, P. R. (2002). *The Molecular Biology of Human Caliciviruses* (pp. 180–196). John Wiley & Sons, Ltd. <https://doi.org/10.1002/0470846534.ch11>
11. Costantini, V., Loisy, F., Joens, L., Le Guyader, F. S., & Saif, L. J. (2006). Human and Animal Enteric Caliciviruses in Oysters from Different Coastal Regions of the United States. *Applied and Environmental Microbiology*, 72(3), 1800–1809. <https://doi.org/10.1128/AEM.72.3.1800-1809.2006>

12. da Silva Poló, T., Peiró, J. R., Mendes, L. C. N., Ludwig, L. F., de Oliveira-Filho, E. F., Bucardo, F., ... Mauroy, A. (2017). Human norovirus infection in Latin America. *Journal of Clinical Virology : The Official Publication of the Pan American Society for Clinical Virology*, 78, 111–9. <https://doi.org/10.1016/j.jcv.2017.03.016>
13. Dolin, R., Blacklow, N. R., DuPont, H., Buscho, R. F., Wyatt, R. G., Kasel, J. A., ... Chanock, R. M. (1972). Biological Properties of Norwalk Agent of Acute Infectious Nonbacterial Gastroenteritis. *Experimental Biology and Medicine*, 140(2), 578–583. <https://doi.org/10.3181/00379727-140-36508>
14. Dreyfuss, M. S. (2009). Is Norovirus a Foodborne or Pandemic Pathogen? An Analysis of the Transmission of Norovirus-Associated Gastroenteritis and the Roles of Food and Food Handlers. *Foodborne Pathogens and Disease*, 6(10), 1219–1228. <https://doi.org/10.1089/fpd.2009.0320>
15. Duizer, E., Bijkerk, P., Rockx, B., De Groot, A., Twisk, F., & Koopmans, M. (2004). Inactivation of caliciviruses. *Applied and Environmental Microbiology*, 70(8), 4538–43. <https://doi.org/10.1128/AEM.70.8.4538-4543.2004>
16. Elmnasser, N., Guillou, S., Leroi, F., Orange, N., Bakhrouf, A., & Federighi, M. (2007). Pulsed-light system as a novel food decontamination technology: a review. *Canadian Journal of Microbiology*, 53(7), 813–821. <https://doi.org/10.1139/W07-042>
17. Estes, M. K., Prasad, B. V., & Atmar, R. L. (2006). Noroviruses everywhere: has something changed? *Current Opinion in Infectious Diseases*, 19(5), 467–474. <https://doi.org/10.1097/01.qco.0000244053.69253.3d>
18. Fankhauser, R. L., Monroe, S. S., Noel, J. S., Humphrey, C. D., Bresee, J. S., Parashar, U. D., ... Glass, R. I. (2002). Epidemiologic and Molecular Trends of “Norwalk-like Viruses” Associated with Outbreaks of Gastroenteritis in the United States. *The Journal of Infectious Diseases*, 186(1), 1–7. <https://doi.org/10.1086/341085>
19. Fankhauser, R. L., Noel, J. S., Monroe, S. S., Ando, T., Glass, R. I., & Frankhauser R Noel J, M. S. A. T. G. R. (1998). Molecular Epidemiology of “Norwalk-like Viruses” in outbreaks of gastroenteritis in the United States. *J. Infect. Dis.*, 178(6), 1571–1578. <https://doi.org/10.1086/314525>
20. Farkas, T., Cross, R. W., Hargitt, E., Lerche, N. W., Morrow, A. L., & Sestak, K. (2010). Genetic Diversity and Histo-Blood Group Antigen Interactions of Rhesus Enteric Caliciviruses. *Journal of Virology*, 84(17), 8617–8625. <https://doi.org/10.1128/JVI.00630-10>
21. Gallimore, C. I., Green, J., Lewis, D., Richards, A. F., Lopman, B. A., Hale, A. D., ... Brown, D. W. G. (2004). Diversity of noroviruses cocirculating in the north of England from 1998 to 2001. *Journal of Clinical Microbiology*, 42(4), 1396–401. <https://doi.org/10.1128/JCM.42.4.1396-1401.2004>
22. Godoy, P., Domínguez, A., Alvarez, J., Camps, N., Barrabeig, I., Bartolomé, R., ... grupo de estudio de gastroenteritis víricas en Cataluña. (n.d.). [Norovirus outbreaks in hospitals and nursing homes in Catalonia, Spain]. *Revista Espanola de Salud Publica*, 83(5), 745–50. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/20111822>
23. Graciela Gutierrez. (n.d.). Solving a 48-year-old mystery: scientists succeed at growing noroviruses in human intestinal cell cultures in the lab | Baylor College of Medicine | Houston, Texas. Retrieved February 3, 2017, from <https://www.bcm.edu/news/molecular-virology-and-microbiology/norovirus-infection-grown-in-intestinal-cells>
24. Hall, A. J., Vinjé, J., Lopman, B., Park, G. W., Yen, C., Gregoricus, N., & Parashar, U. (2011). Updated Norovirus Outbreak Management and Disease Prevention Guidelines. *Morbidity and Mortality Weekly Report (MMWR) Recommendations and Reports*, 60(RR03), 1–15.
25. Hardy, M. E., Kramer, S. F., Treanor, J. J., & Estes, M. K. (1997). Human calicivirus genogroup II capsid sequence diversity revealed by analyses of the prototype Snow Mountain agent. *Archives of Virology*, 142(7), 1469–1479. <https://doi.org/10.1007/s007050050173>
26. Ho, E. C. M., Cheng, P. K. C., Lau, A. W. L., Wong, A. H., & Lim, W. W. L. (2007). Atypical norovirus epidemic in Hong Kong during summer of 2006 caused by a new genogroup II/4 variant. *Journal of Clinical Microbiology*, 45(7), 2205–11. <https://doi.org/10.1128/JCM.02489-06>
27. Iturriza-Gómara, M., & Lopman, B. (2014). Norovirus in healthcare settings. *Current Opinion in Infectious Diseases*, 27(5), 437–43. <https://doi.org/10.1097/QCO.0000000000000094>
28. Jiang, X., Matson, D. O., Cubitt, W. D., & Estes, M. K. (1996). Genetic and antigenic diversity of human caliciviruses (HuCVs) using RT-PCR and new EIAs. *Arch.Virol.Suppl*, 12, 251–262.
29. Jiang, X., Matson, D. O., Ruiz-Palacios, G. M., Hu, J., Treanor, J., & Pickering, L. K. (1995). Expression, self-assembly, and antigenicity of a snow mountain agent-like calicivirus capsid protein. *Journal of Clinical Microbiology*, 33(6), 1452–5. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/7650166>
30. Kageyama, T., Kojima, S., Shinohara, M., Uchida, K., Fukushi, S., Hoshino, F. B., ... Katayama, K. (2003). Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. *J Clin Microbiol*, 41(4), 1548–1557. <https://doi.org/10.1128/JCM.41.4.1548>

31. Kapikian, A. Z., Wyatt, R. G., Dolin, R., Thornhill, T. S., Kalica, A. R., & Chanock, R. M. (1972). Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis. *Journal of Virology*, 10(5), 1075–81. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/4117963>
32. Kele, B., Lengyel, G., & Deak, J. (2011). Comparison of an ELISA and two reverse transcription polymerase chain reaction methods for norovirus detection. *Diagnostic Microbiology and Infectious Disease*, 70(4), 475–478. <https://doi.org/10.1016/j.diagmicrobio.2011.04.002>
33. Kiraz, N., Samasti, M., & Aygün, G. (2011). Mikrobiyoloji ve klinik mikrobiyoloji ders kitabı. Istanbul Universitesi.
34. Koch, J., Schneider, T., Stark, K., & Schreier, E. (2006). Norovirusinfektionen in Deutschland. *Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz*, 49(3), 296–309. <https://doi.org/10.1007/s00103-006-1231-x>
35. Koo, H. L., Ajami, N., Atmar, R. L., & DuPont, H. L. (2010). Noroviruses: The leading cause of gastroenteritis worldwide. *Discovery Medicine*, 10(50), 61–70. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/20670600>
36. Koopmans, M., & Duizer, E. (2004). Foodborne viruses: an emerging problem. *International Journal of Food Microbiology*, 90(1), 23–41. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/14672828>
37. Koopmans, M., Kapikian, A. Z., Teunis, P. F., Moe, C. L., Liu, P., al., et, ... Goldmann, D. A. (2009). Noroviruses in healthcare settings: a challenging problem. *The Journal of Hospital Infection*, 73(4), 331–7. <https://doi.org/10.1016/j.jhin.2009.06.028>
38. Kroneman, A., Verhoef, L., Harris, J., Vennema, H., Duizer, E., van Duynhoven, Y., ... Koopmans, M. (2008). Analysis of Integrated Virological and Epidemiological Reports of Norovirus Outbreaks Collected within the Foodborne Viruses in Europe Network from 1 July 2001 to 30 June 2006. *Journal of Clinical Microbiology*, 46(9), 2959–2965. <https://doi.org/10.1128/JCM.00499-08>
39. Lew, J. F., Kapikian, A. Z., Valdesuso, J., & Green, K. Y. (1994). Molecular characterization of Hawaii virus and other Norwalk-like viruses: evidence for genetic polymorphism among human caliciviruses. *The Journal of Infectious Diseases*, 170(3), 535–42. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/8077710>
40. Lew, J. F., Petric, M., Kapikian, A. Z., Jiang, X., Estes, M. K., & Green, K. Y. (1994). Identification of minireovirus as a Norwalk-like virus in pediatric patients with gastroenteritis. *Journal of Virology*, 68(5), 3391–6. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/8151799>
41. Lopman, B. A., Reacher, M. H., Van Duynhoven, Y., Hanon, F. X., Brown, D., & Koopmans, M. (2003). Viral gastroenteritis outbreaks in Europe, 1995-2000. *Emerging Infectious Diseases*, 9(1), 90–96. <https://doi.org/10.3201/eid0901.020184>
42. Lopman, B., Gastañaduy, P., Park, G. W., Hall, A. J., Parashar, U. D., & Vinjé, J. (2012). Environmental transmission of norovirus gastroenteritis. *Current Opinion in Virology*, 2(1), 96–102. <https://doi.org/10.1016/j.coviro.2011.11.005>
43. Lopman, B., Zambon, M., & Brown, D. W. (2008). The Evolution of Norovirus, the “Gastric Flu.” *PLoS Medicine*, 5(2), e42. <https://doi.org/10.1371/journal.pmed.0050042>
44. Maccannell, T., Umscheid, C. A., Agarwal, R. K., Lee, I., Kuntz, G., Stevenson, K. B., & Ga, A. (2011). Updated norovirus outbreak management and disease prevention guidelines. *MMWR Recomm Rep*.
45. Magulski, T., Paulmann, D., Bischoff, B., Becker, B., Steinmann, E., Steinmann, J., ... Steinmann, J. (2009). Inactivation of murine norovirus by chemical biocides on stainless steel. *BMC Infectious Diseases*, 9(1), 107. <https://doi.org/10.1186/1471-2334-9-107>
46. Ozawa, K., Oka, T., Takeda, N., & Hansman, G. S. (2007). Norovirus infections in symptomatic and asymptomatic food handlers in Japan. *Journal of Clinical Microbiology*, 45(12), 3996–4005. <https://doi.org/10.1128/JCM.01516-07>
47. Öztürk, R. (2008). Enfeksiyon hastalıkları ve mikrobiyolojisi. (A. W. Topçu, G. Söyletir, & M. Doğanay, Eds.) (3rd ed.). Nobel Tıp Kitabevleri.
48. Parashar, U., Quiroz, E. S., Mounts, A. W., Monroe, S. S., Fankhauser, R. L., Ando, T., ... Glass, R. I. (2001). “Norwalk-like viruses”: Public health consequences and outbreak management. *MMWR. Recommendations and Reports : Morbidity and Mortality Weekly Report. Recommendations and Reports*, 50(RR-9), 1–17. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/15580799>
49. Parra, G. I., Abente, E. J., Sandoval-Jaime, C., Sosnovtsev, S. V., Bok, K., & Green, K. Y. (2012). Multiple Antigenic Sites Are Involved in Blocking the Interaction of GII.4 Norovirus Capsid with ABH Histo-Blood Group Antigens. *Journal of Virology*, 86(13), 7414–7426. <https://doi.org/10.1128/JVI.06729-11>
50. Patel, M. M., Hall, A. J., Vinjé, J., Parashar, U. D., Bryce, J., Boschi-Pinto, C., ... Ward, R. L. (2009). Noroviruses: a comprehensive review. *Journal of Clinical Virology : The Official Publication of the Pan American Society for Clinical Virology*, 44(1), 1–8. <https://doi.org/10.1016/j.jcv.2008.10.009>
51. Percival, S. L., Yates, M. V. (Marylynn V. ), Williams, D. W., Chalmers, R., & Gray, N. F. (2004). *Microbiology of waterborne diseases : microbiological aspects and risks.*

52. Rabenau, H. F., Stürmer, M., Buxbaum, S., Walczok, A., Preiser, W., & Doerr, H. W. (2003). Laboratory Diagnosis of Norovirus: Which Method Is the Best? *Intervirology*, 46(4), 232–238. <https://doi.org/10.1159/000072433>
53. Reintjes, R., Thelen, M., Reiche, R., & Csohan, A. (2007). Benchmarking national surveillance systems: a new tool for the comparison of communicable disease surveillance and control in Europe. *The European Journal of Public Health*, 17(4), 375–380. <https://doi.org/10.1093/eurpub/ckl256>
54. Richards, A. , Lopman, B., Gunn, A., Curry, A., Ellis, D., Cotterill, H., ... Brown, D. W. . (2003). Evaluation of a commercial ELISA for detecting Norwalk-like virus antigen in faeces. *Journal of Clinical Virology*, 26(1), 109–115. [https://doi.org/10.1016/S1386-6532\(02\)00267-6](https://doi.org/10.1016/S1386-6532(02)00267-6)
55. Richards, G. P. (1999). Limitations of molecular biological techniques for assessing the virological safety of foods. *Journal of Food Protection*, 62(6), 691–7. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/10382664>
56. Richards, G. P. (2012). Critical review of norovirus surrogates in food safety research: rationale for considering volunteer studies. *Food and Environmental Virology*, 4(1), 6–13. <https://doi.org/10.1007/s12560-011-9072-7>
57. Rockx, B., de Wit, M., Vennema, H., Vinjé, J., de Bruin, E., van Duynhoven, Y., & Koopmans, M. (2002). Natural history of human calicivirus infection: a prospective cohort study. *Clinical Infectious Diseases*, 35(3), 246–253. <https://doi.org/10.1086/341408>
58. Said, M. A., Perl, T. M., & Sears, C. L. (2008). Gastrointestinal Flu: Norovirus in Health Care and Long-Term Care Facilities on JSTOR. *Clinical Infectious Diseases*, 47(9), 1202–1208.
59. Said, M. A., Perl, T. M., Sears, C. L., & Sears, C. L. (2008). Healthcare Epidemiology: Gastrointestinal Flu: Norovirus in Health Care and Long-Term Care Facilities. *Clinical Infectious Diseases*, 47(9), 1202–1208. <https://doi.org/10.1086/592299>
60. Scallan, E., Griffin, P. M., Angulo, F. J., Tauxe, R. V., & Hoekstra, R. M. (2011). Foodborne Illness Acquired in the United States—Unspecified Agents. *Emerging Infectious Diseases*, 17(1), 16–22. <https://doi.org/10.3201/eid1701.P21101>
61. Sickbertbenett, E., Weber, D., Gergenteague, M., Sobsey, M., Samsa, G., & Rutala, W. (2005). Comparative efficacy of hand hygiene agents in the reduction of bacteria and viruses. *American Journal of Infection Control*, 33(2), 67–77. <https://doi.org/10.1016/j.ajic.2004.08.005>
62. Teunis, P. F. M., Moe, C. L., Liu, P., E. Miller, S., Lindesmith, L., Baric, R. S., ... Calderon, R. L. (2008). Norwalk virus: How infectious is it? *Journal of Medical Virology*, 80(8), 1468–1476. <https://doi.org/10.1002/jmv.21237>
63. Thornton, A. C., Jennings-Conklin, K. S., & McCormick, M. I. (2004). Noroviruses: agents in outbreaks of acute gastroenteritis. *Disaster Management & Response*, 2(1), 4–9. <https://doi.org/10.1016/j.dmr.2003.11.001>
64. Thornton, A., Jennings-Conklin, K. S., & McCormick, M. I. (2004). Noroviruses: Agents in outbreaks of acute gastroenteritis. *Disaster Management and Response*. <https://doi.org/10.1016/j.dmr.2003.11.001>
65. Todd, E. C. D., Greig, J. D., Bartleson, C. A., & Michaels, B. S. (2009). Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 6. Transmission and survival of pathogens in the food processing and preparation environment. *Journal of Food Protection*, 72(1), 202–19. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/19205488>
66. Treanor, J. J., & Dolin, R. (2000). Norwalk virus and other caliciviruses. *Principles and Practice of Infectious Diseases*, 5, 1949–1956.
67. Ustaçelebi, Ş., Abacıoğlu, H., Badur, S. (2004). Moleküler, Klinik ve Tanısal Viroloji. (A. D. US & K. Ergüner, Eds.). Bilimsel Tıp Yayınevi.
68. Uyar, Y., Carhan, A., Ozkaya, E., & Ertek, M. (2008). [Evaluation of laboratory diagnosis of the first norovirus outbreak in Turkey in 2008]. *Mikrobiyoloji Bulteni*, 42(4), 607–15. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/19149082>
69. Verhoef, L., Vennema, H., van Pelt, W., Lees, D., Boshuizen, H., Henshilwood, K., ... Food-Borne Viruses in Europe Network. (2010). Use of Norovirus Genotype Profiles to Differentiate Origins of Foodborne Outbreaks. *Emerging Infectious Diseases*, 16(4), 617–624. <https://doi.org/10.3201/eid1604.090723>
70. Westrell, T., Dusch, V., Ethelberg, S., Harris, J., Hjertqvist, M., Jourdan-da Silva, N., Vold, L. (2010). Norovirus outbreaks linked to oyster consumption in the United Kingdom, Norway, France, Sweden and Denmark, 2010. *Euro Surveillance : Bulletin European Sur Les Maladies Transmissibles = European Communicable Disease Bulletin*, 15(12). Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/20350499>
71. Widdowson, M.-A., Sulka, A., Bulens, S. N., Beard, R. S., Chaves, S. S., Hammond, R., Glass, R. I. (2005). Norovirus and foodborne disease, United States, 1991-2000. *Emerging Infectious Diseases*, 11(1), 95–102. <https://doi.org/10.3201/eid1101.040426>
72. Wobus, C. E., Karst, S. M., Thackray, L. B., Chang, K.-O., Sosnovtsev, S. V., Belliot, G., IV. (2004). Replication of Norovirus in cell culture reveals a tropism for dendritic cells and macrophages. *PLoS Biology*, 2(12), e432. <https://doi.org/10.1371/journal.pbio.0020432>

73. Yılmaz, A., Bostan, K., Altan, E., Muratoglu, K., Turan, N., Tan, D., Yılmaz, H. (2011). Investigations on the Frequency of Norovirus Contamination of Ready-to-Eat Food Items in Istanbul, Turkey, by Using Real-Time Reverse Transcription PCR. *Journal of Food Protection*, 74(5), 840–843. <https://doi.org/10.4315/0362-028X.JFP-10-475>.

**How to cite this article**

ÇÖL, B. G., & YIKMIŞ, S. (2017). Human Norovirus Infection: Identification, Epidemics and Treatment. *Int. J. Agr. Life. Sci*, 3(1), 147-156. doi: 10.22573/spg.ijals.017.s12200079.

**CONFLICTS OF INTEREST**

“The authors declare no conflict of interest”.

© 2017 by the authors; licensee SKY FOX Publishing Group, Tamilnadu, India. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>).